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(21) International Application Number: PCT/US99/16577 (22) International Filing Date: 22 July 1999 (22.07.99) (30) Priority Data: 09/121,921 24 July 1998 (24.07.98) US (71) Applicant (for all designated States except US): CORVAS INTERNATIONAL, INC. [US/US]; 3030 Science Park Road, San Diego, CA 92121 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BRUNCK, Terence, K. [US/US]; 4949 Quincy, San Diego, CA 92109 (US). TAMURA, Susan, Y. [US/US]; 8997 Gainsborough, San Diego, CA 92129 (US). (74) Agents: BIGGS, Suzanne, L. et al.; Lyon & Lyon LLP, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: INHIBITORS OF UROKINASE AND BLOOD VESSEL FORMATION (57) Abstract Novel compounds having activity inhibitors of urokinase and in reducing or inhibiting blood vessel formation are provided. These compounds have an arginine or arginine mimic aldehyde or an arginine ketoamide group at P1. These compounds are useful <i>in vitro</i> for monitoring plasminogen activator levels and <i>in vivo</i> in treatment of conditions which are ameliorated by inhibition of or decreased activity of urokinase and in treating pathologic conditions wherein blood vessel formation is related to a pathologic condition.		

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DESCRIPTIONInhibitors of Urokinase and Blood Vessel Formation5 Cross Reference to Related Applications

The application is a continuation-in-part of commonly-assigned and copending United States Serial No. 09/121,921, filed July 24, 1998, the disclosure of which is incorporated herein by reference.

10 Field Of The Invention

Urokinase is an enzyme involved in the metastasis of tumor cells, neovascularization, and other activities. One purpose of the present invention is to provide novel compounds which are active as inhibitors of urokinase that
15 can be used to inhibit the activity of urokinase and thereby attenuate its deleterious effects. Another purpose of the present invention is to provide novel compounds which inhibit blood vessel formation, particularly blood vessel formation related to a pathologic condition.

20 Background And Introduction To The Invention

Urinary-type plasminogen activator (uPA; urokinase) is a serine protease within the trypsin/chymotrypsin family. In its physiological state, uPA is found in three forms: single chain pro-uPA, two chain uPA, and low molecular weight uPA
25 (lacks N-terminal domains). The zymogen, pro-uPA, is converted to u-PA by cleavage of the peptide bond at K158-I159. The resultant two chain uPA is linked by disulfide

bridges, has an M_r of about 50 kD, and a C-terminal serine proteinase domain.

The activity of uPA is focused to cell surfaces upon binding to its receptor, uPAR. uPAR is a single-chain glycosyl phosphatidyl inositol (GPI)-anchored membrane receptor. The N-terminal 92 amino acids of uPAR play a dominant role in binding to uPA and pro-uPA. Receptor for uPA has been located on T-cells, NK cells, monocytes, and neutrophils, as well as vascular endothelial cells, fibroblasts, smooth muscle cells, keratinocytes, placental trophoblasts, hepatocytes, and a wide variety of tumor cells.

After conversion of pro-uPA to uPA, which occurs primarily at the uPAR on the cell surface, uPA activates plasminogen to plasmin. Activation occurs upon cleavage at residues PGR-VV for human plasminogen, or at residues SGR-IV for bovine plasminogen. Because plasminogen also is present on the cell surface, this activation cascade focuses the activity of u-PA and plasmin on the plasma membrane. Plasmin has many roles, including activation of additional uPA and other enzymes, digestion of fibrin, and digestion of components of the extracellular matrix (ECM). Digestion of the ECM surrounding a tumor removes the ECM as a physical barrier to metastasizing cells, which are then free to leave primary tumors and invade secondary sites. A review of the role of the uPA/uPAR system in cancer metastasis is provided in "The Urokinase-type Plasminogen Activator System in Cancer Metastasis: A Review", Andreasen et al., Int. J. Canc. 72:1-22 (1997).

A correlation between a high level of uPA and a high rate of metastasis, and poor prognosis, has been noted in certain tumors, especially breast cancer [Quax et al., J. Cell Biol. 115:191-199 (1991); Duffy et al., Cancer Res.

50:6827-6829 (1990)]. For instance, tumors of the lung [Oka et al., Cancer Res. 51:3522-3525 (1991)], bladder [Hasui et al., Int. J. Cancer 50:871-873 (1992)], stomach [Nekarda et al., Lancet 343:117 (1994)], cervical cancer [Kobayashi et al., Cancer Res. 54:6539-6548 (1994)], ovary [Kuhn et al., Gynecol. Oncol. 55:401-409 (1994)], kidney [Hofmann et al., Cancer 78:487-492 (1996)], brain [Bindahl et al., J. Neuro-Oncol. 22:101-110 (1994)], and soft tissue sarcoma [Choong et al., Int. J. Cancer (Pred. Oncol.) 69:268-272 (1996)] have exhibited a high level of uPA and/or uPA activity and a high rate of metastases. Overproduction of uPA has been reported to result in increased skeletal metastasis by prostate cancer cells in vivo [Achbarou et al., Cancer Res. 54:2372-2377 (1994)]

15 Inhibition or lowering of uPA activity, or disruption/inhibition of the interaction between uPA and its receptor (uPAR) has been shown to have a positive effect on maintenance of the extracellular matrix and an inhibitory effect on metastasis [Ossowski and Reich, Cell 35:611-619 (1983); Ossowski, Cell 52:321-328 (1988); Ossowski, J. Cell Biol. 107:2437-2445 (1988); Wilhelm et al., Clin. Exp. Metastasis 13:296-302 (1995); Achbarou et al., Cancer Res. 54:2372-2377 (1994); Crowley et al., Proc. Natl. Acad. Sci. USA 90:5021-5025 (1993); Kook et al., EMBO J. 13:3983-3991 (1994)]. The results of such experimental studies suggest that uPA-catalyzed plasminogen activation is rate-limiting for tumor progression, local tumor invasion and/or formation of distant metastasis. [Andreasen et al., Int. J. Canc. 72:1-22 (1997)].

30 The effects of the uPA system on cell migration and invasion are thought to be due to both a proteolytic effect of plasmin-mediated degradation of the extracellular matrix,

as well as more a direct interaction of the uPA receptor with components of the extracellular matrix. Degradation of the extracellular matrix permits a metastasizing cell to invade the matrix, whereas interaction between uPA receptor and the matrix itself assists a cell in its migration. Localization of the uPA/plasmin system on the cell surface, or the leading edge of metastasizing cells, is consistent with postulated role of uPA in metastasis [Plesner et al., Stem Cells 15:398-408 (1997)].

Interaction of uPAR with vitronectin, a component of the extracellular matrix, mediates cell adhesion and can be enhanced when uPAR is bound by uPA. Cell surface adhesion molecules, integrins, also appear to be involved in this adhesion function, particularly beta-1 and beta-2 integrins [Paysant et al., Br. J. Haematol. 100:45-51 (1998); Simon et al., Blood 88:3185-3194 (1996)]. The CD11b/CD18 integrin can associate with the uPA-uPAR complex and promote adhesion of cells bearing these receptors, e.g., neutrophils, leukocytes.

The uPA/uPAR system also is involved in the establishment of new vasculature, or neovascularization.

Establishment of new vasculature is required for sustaining primary and metastatic tumor growth. Pathological neovascularization also is a characteristic of retinal disease, rubeosis iritis, proliferative vitreo retinopathy inflammatory disease, diabetic retinopathy, chronic uveitis, Fuch's heterochromic iridocyclitis, neovascular glaucoma, corneal or optic nerve neovascularization, vascular disease, pterygium, glaucoma surgery bleb failure, hyperkeratosis, cheloid and polyp formation (see EP 451,130). Undesired angiogenesis also can occur in the following conditions or can be a result of the following activities: macular degeneration, retinopathy of prematurity, corneal graft

rejection, retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sogrens disease, acne rosacea, phlyectenulosis, syphilis, Mycobacteria infections other than leprosy, lipid degeneration, chemical burns, bacterial or fungal ulcers, Herpes simplex or zoster infections, protozoan infections, Kaposi's sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegeners sarcoidosis, sleritis, Steven's Johnson disease, radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein or artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular tissue, hemangiomas, Osler-Wever-Rendu, solid tumors, blood borne tumors, AIDS, ocular neovascular disease, osteoarthritis, chronic inflammation, Crohn's disease, ulceritive colitis, tumors of rhabdomyosarcoma, tumors of retinoblastoma, tumors of Ewing sarcoma, tumors of neuroblastoma, tumors of osteosarcoma, leukemia, psoriasis, atherosclerosis, pemphigoid, as recited in U.S. Patent No. 5,712,291.

An antagonist of uPA/uPAR binding (EGF-like domain of uPA fused to Fc of IgG) was said to inhibit neovascularization and growth of the murine B16 melanoma. [Min et al., Cancer Res. 56:2428-2433 (1996)]. Consistent with this finding is the correlation noted between microvessel density, vascular invasion and uPA levels in

breast carcinomas [Hildenbrand et al., Brit. J. Cancer
72:818-823 (1995)]. The known uPA inhibitor amiloride also
was said to inhibit a variety of neovascularization
pathologies [Glaser et al., EP 451,130; Avery et al., Arch.
5 Ophthalmol. 108:1474-1476 (1990)].

There are two primary physiological inhibitors of uPA,
PAI-1 and PAI-2, which are members of the serpin family of
proteinase inhibitors. The binding of serpins to their
cognate proteases involves a large number of interactions
10 between amino acids of each protein, including those in the
serpin reactive loop (Ser-Ala-Arg-Met-Ala (SEQ. ID. NO. 1)
for PAI-1, Thr-Gly-Arg-Thr-Gly (SEQ. ID. NO. 2) for PAI-2).
Introduction of exogenous PAI-2 into experimental animals was
reported to inhibit the rate of lung metastasis [Evans and
15 Lin, Amer. Surg. 61:692-697 (1995); Mueller et al., Proc.
Natl. Acad. Sci. USA 92:205-209 (1995)]. The ability of
PAI-1 to inhibit metastasis has not yet been consistently
shown. The gene for PAI-1, and means for its recombinant
expression, are disclosed in Loskutoff et al., U.S. Patent
20 No. 4,952,512. Recombinant and native human PAI-2 is
disclosed in Stephens et al., U.S. Patent No. 5,422,090.

The most widely studied uPA inhibitors may be within the
4-substituted benzo[b]thiophene-2-carboxamidine class of
inhibitors, of which B428 (4-iodo-benzo[b]thiophene-2-
25 carboxamidine) and B623 are members [Towle et al., Cancer
Res. 53:2553-2559 (1993); Bridges et al., Bioorg. Med. Chem.
1:403-410 (1993); Bridges et al., U.S. Patent No. 5,340,833].
Infusion of B428 in experimental rats inoculated with tumor
cells was said to inhibit uPAR gene expression, decrease the
30 primary tumor volume and decrease metastases [Xing et al.,
Cancer Res. 57:3585-3593 (1997)]. Daily intraperitoneal
treatment of mice bearing tumors with B428 or B623 was said

to block metastasis to muscle and fat, but did not inhibit tumor-induced angiogenesis or reduce the rate of spontaneous lung metastasis. In fact, B623 enhanced the formation of lung metastasis (Alonso et al., Breast Cancer Res. Treat. 40:209-223 (1996)). Infusion of B428 in a syngeneic model of rat prostate cancer also lead to a decrease in primary tumor volume and tumor weight, and a decrease in metastasis [Rabbani et al., Int. J. Cancer 63:840-845 (1995)].

Other known inhibitors of uPA include

10 p-aminobenzamidine, which is a competitive inhibitor of uPA, and amiloride. Both compounds have been shown to reduce tumor size in experimental animals [Jankan et al., Cancer Res. 57:559-563 (1997); Billstrom et al., Int. J. Cancer 61:542-547 (1995)]. Recently, epigallo-cathecin-3 gallate

15 (EGCG), a polyphenol found in green tea, was reported to bind uPA and inhibit its activity [Jankun et al., Nature 387:561 (1997)]. Those researchers concluded EGCG is a weaker inhibitor of uPA than amiloride, but suggested EGCG can be consumed in much higher doses than amiloride without toxic

20 effect. A competitive inhibitor of uPA, α -N-benzylsulfonyl-p-aminophenylalanine, is disclosed by Pye et al. in U.S. Patent No. 4,165,258.

Other approaches at inhibiting the uPA/uPAR system include development of a bifunctional hybrid molecule

25 consisting of the uPAR-binding domain of uPA and PAI-2, which is said to inhibit uPA and bind uPAR in vitro [Ballance et al., Eur. J. Biochem. 207:177-183 (1992)]. Antagonists of uPAR also have been studied [Doyle and Rosenberg, U.S. Patent No. 5,656,726; Min et al., Cancer Res. 56:2428-2433 (1996)],

30 as have antisense oligonucleotides complementary to uPA [Wilhelm et al., Clin. Exp. Metast. 13:296-302 (1995); Iversen and Scholar, U.S. Patent No. 5,552,390]. Antibodies

directed against uPAR, and said to inhibit the binding of uPA to uPAR, are disclosed by Dano et al. in U.S. Patent No. 5,519,120. Small molecules said to inhibit urokinase, along with a variety of other serine proteases, include those disclosed by Abe et al. in U.S. Patent No. 5,508,385 and U.S. Patent No. 5,153,176, and by Takano et al. in J. Pharmacol. Exp. Therapeut. 271:1027-1033 (1994).

Compounds have been developed to directly inhibit the binding of u-PA to uPAR (Crowley et al., Proc. Natl. Acad. Sci. USA 90:5021-5025 (1993); Goodson et al., Proc. Natl. Acad. Sci. USA 91:7129-7133 (1994); Kobayashi et al., Brit. J. Cancer 67:537-544 (1993), and Int. J. Cancer 57:727-73f3 (1994), and J. Biol. Chem. 270:8361-8366 (1995); Lu et al., FEBS Lett. 356:56-59 (1994) and FEBS Lett. 380:21-24 (1996)).

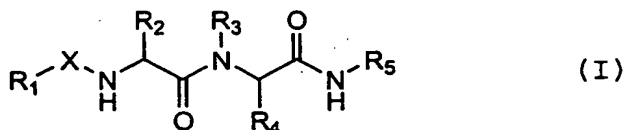
Additionally, pro-hepatocyte growth factor (HGF), a cell migration stimulating protein, is a substrate of uPA [Naldin et al., EMBO J. 11:4825-4833 (1992)]. Direct cleavage of a 66kDa extracellular matrix protein and fibronectin by uPA also has been reported, which suggests a more direct role for uPA in facilitating cell migration [Quigley et al., Proc. Natl. Acad. Sci. 84:2776-2780 (1987)]. Thus, inhibition of uPA may affect these activities, as well.

Summary Of The Invention

The present invention is directed to novel peptide aldehyde and ketoamide compounds. The peptide aldehyde compounds have an arginine or arginine mimic at P1. The ketoamide compounds have an arginine ketoamide group at P1. These compounds have activity as potent inhibitors of urokinase and thereby are useful in decreasing its deleterious effects. Compounds of the present invention are

active in inhibiting blood vessel formation, particularly that related to a pathologic process.

Thus in one aspect, the present invention is directed to compounds of the formula (I):



wherein:

(a) X is selected from the group consisting of $\text{-S(O)}_2\text{-}$, $\text{-N(R')-S(O)}_2\text{-}$, -(C=O)- , -OC(=O)- , -NH-C(=O)- , -P(O)(R')- , and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with the proviso that when X is -P(O)(R')- , then R' is not hydrogen;

(b) R₁ is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms which is optionally substituted with Y₁,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon atoms which is optionally mono-, di- or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2,

5 including, $-\text{N} \begin{array}{c} \text{---} \end{array} \text{V}$ wherein $-\text{N} \begin{array}{c} \text{---} \end{array} \text{V}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y₁, Y₂, and/or Y₃,

(6) alkenyl of 2 to about 6 carbon atoms which is
10 optionally substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y₁, Y₂,
15 and/or Y₃,

(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-
20 substituted with Y₁, Y₂, and/or Y₃,

(9) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y₁, Y₂, and/or Y₃,

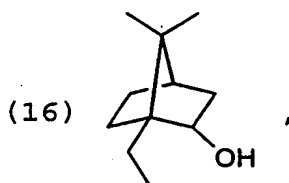
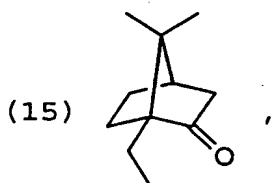
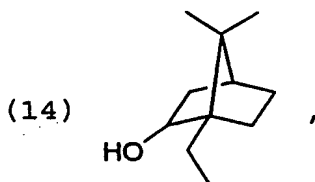
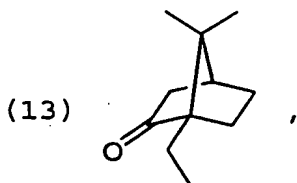
(10) heteroaralkyl of about 5 to about 14 ring
25 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y₁,
30 Y₂, and/or Y₃,

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(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,

10



(17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms;

(18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(19) perfluoroaryl of about 6 to about 14 carbon atoms,

(20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(21) hydrogen when X is a direct link;

wherein each Y_1 , Y_2 , and Y_3 is independently selected and is

5 (i) selected from the group consisting of halogen, cyano, nitro, tetrazolyl, guanidino, amidino, methylguanidino, $-\text{CF}_3$, $-\text{CF}_2\text{CF}_3$, $-\text{CH}(\text{CF}_3)_2$, $-\text{C}(\text{OH})(\text{CF}_3)_2$, $-\text{OCF}_3$, $-\text{OCF}_2\text{H}$, $-\text{OCF}_2\text{CF}_3$, $-\text{OC}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{NHZ}_1$, $-\text{OC}(\text{O})\text{NZ}_1\text{Z}_2$, $-\text{NHC}(\text{O})\text{Z}_1$, $-\text{NHC}(\text{O})\text{NH}_2$, $-\text{NHC}(\text{O})\text{NZ}_1$, $-\text{HC}(\text{O})\text{NZ}_1\text{Z}_2$, $-\text{C}(\text{O})\text{OH}$,
 10 $-\text{C}(\text{O})\text{OZ}_1$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{NHZ}_1$, $-\text{C}(\text{O})\text{NZ}_1\text{Z}_2$, $-\text{P}(\text{O})_3\text{H}_2$, $-\text{P}(\text{O})_3(\text{Z}_1)_2$, $-\text{S}(\text{O})_3\text{H}$, $-\text{S}(\text{O})_m\text{Z}_1$, $-\text{Z}_1$, $-\text{OZ}_1$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NHZ}_1$, $-\text{NZ}_1\text{Z}_2$, $-\text{N-morpholino}$, $-\text{S}(\text{CF}_2)_q\text{CF}_3$, and $-\text{S}(\text{O})_m(\text{CF}_2)_q\text{CF}_3$,
 wherein m is 0, 1 or 2, q is an integer from 0 to 5, and Z_1 and Z_2 are independently selected from the group consisting
 15 of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms, or

(ii) Y_1 and Y_2 are selected together to be
 20 $-\text{O}[\text{C}(\text{Z}_3)(\text{Z}_4)]_r\text{O}-$, wherein r is an integer from 1 to 4 and Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon
 25 atoms, and heteroaralkyl of about 5 to about 14 ring atoms;

(c) R_2 is selected from the group consisting of H, $-\text{CH}_3$, $-\text{C}_2\text{H}_5$, $-(\text{CH}_2)_2\text{OH}$, $-(\text{CH}_2)_2\text{OA}_2$, $-\text{CH}(\text{R}_6)\text{OH}$, $-\text{CH}(\text{R}_6)\text{OA}_2$ and $-\text{CH}_2\text{NH}-\text{X}'-\text{R}_6$ wherein A_2 is $-\text{C}(=\text{O})\text{OR}_9$ or $-\text{C}(=\text{O})\text{R}_9$; X' is
 30 selected from the group consisting of $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2-\text{N}(\text{R}'')$, $-(\text{C}=\text{O})-$, $-\text{C}(=\text{O})-\text{O}-$, $-\text{C}(=\text{O})-\text{NH}-$, $-\text{P}(\text{O})(\text{R}'')$, and a direct link, wherein R'' is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of

about 7 to about 16 carbon atoms with the proviso that when X' is -P(O)(R'')-, then R'' is not hydrogen; R₆ is selected from the group consisting of:

- (1) alkyl of 1 to about 12 carbon atoms,
5 optionally substituted with Y₁,
- (2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,
- 10 (3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y₁, Y₂, and/or Y₃,
- (4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms,
15 wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,
- (5) heterocyclo of 4 to about 10 ring atoms with
20 the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including $\text{--}\overset{\text{N}}{\bigcirc}\text{--}$, wherein $\text{--}\overset{\text{N}}{\bigcirc}\text{--}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-,
25 -S(=O)-, -S(O)₂- or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y₁, Y₂, and/or Y₃,
- (6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-
30 substituted on the ring with Y₁, Y₂, and/or Y₃,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

5 (8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

10 (9) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from
15 oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(11) aralkenyl of about 8 to about 15 carbon atoms
20 which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(12) heteroaralkenyl of 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen,
25 and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 , and

(13) hydrogen; and

R_9 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms,
30 optionally substituted with Y_1 ,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon

atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring
5 with Y_1 , Y_2 , and/or Y_3 ,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is
10 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group
15 consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including $-N \bigcirc V$, wherein $-N \bigcirc V$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$, $-S(O)_2-$ or $-S-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1 ,
20 Y_2 , and/or Y_3 ,

(6) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(7) heteroaryl of about 5 to about 14 ring
25 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(8) aralkyl of about 7 to about 15 carbon
30 atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 , and

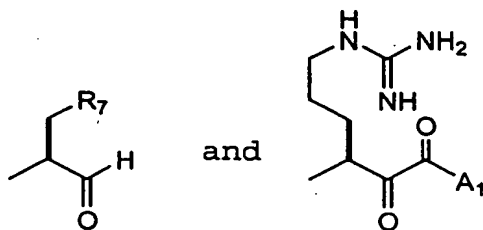
(10) hydrogen, with the proviso that R_9 is not hydrogen when A_2 is $-C(=O)OR_9$;

10 (d) R_3 is selected from H or methyl, or R_3 and R_4 are selected together as set forth in (f);

(e) R_4 is in the S configuration and is selected from the group consisting of H, $-\text{CH}_2-\text{S}-\text{CH}_3$, $-\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CN}$, lower alkyl of 1 to about 3 carbon atoms, $-\text{CH}_2\text{C}\equiv\text{CH}$, $-\text{CH}_2\text{CH}=\text{CH}_2$ and
15 $-\text{CH}=\text{CH}_2$ or R_3 and R_4 are selected together as set forth in (f);

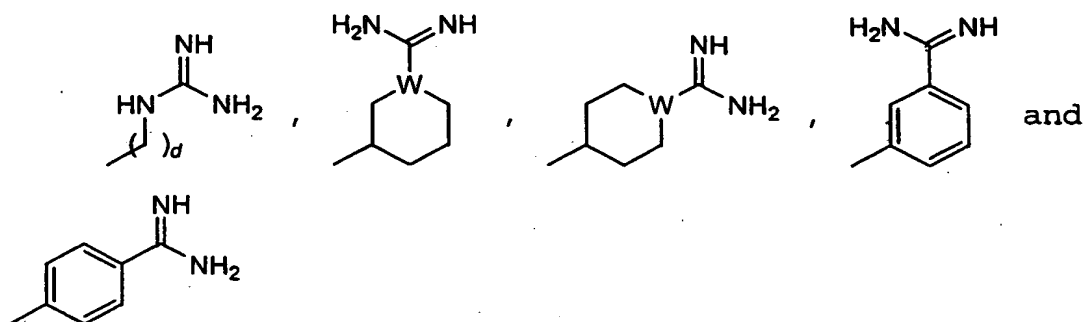
(f) alternatively, R_3 and R_4 are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecholy, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, and
20 3,4-dehydroprolyl;

(g) R_5 is selected from the group consisting of



wherein R_7 is selected from

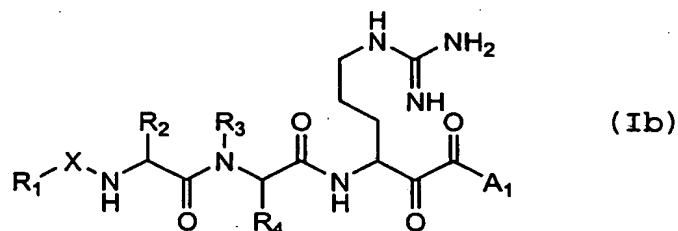
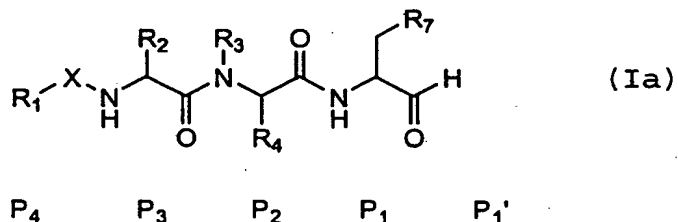
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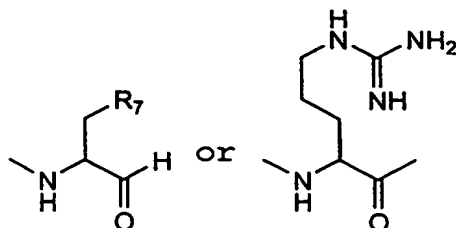
wherein d is an integer from 1 to 3 and W is -N- or -CH-; and

- (h) A₁ is -NHR₈, wherein R₈ is alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 15 carbon atoms, all optionally mono-, di or tri-substituted with Y₁, Y₂ and/or Y₃ or is hydrogen; and pharmaceutically acceptable salts thereof.

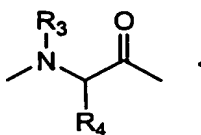
The compounds of the present invention can be divided into parts termed P₁', P₁, P₂, P₃ and P₄ as shown in the following formulas Ia and Ib:



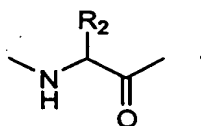
wherein X, R₁, R₂, R₃, R₄, R₇ and A₁ are as defined in connection with formula (I). Thus, the portion of a compound of formula (I) referred to as P₁ or P₁' is the moiety



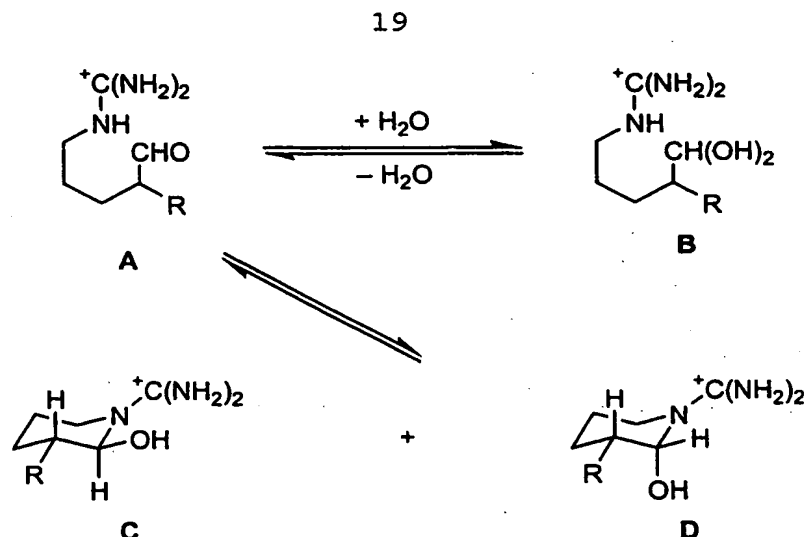
The portion of a compound of formula (I) referred to as P₂ or P₂ is the moiety



- 5 The portion of a compound of formula (I) referred to as P₃ or P₃ is the moiety



Peptidyl arginine aldehydes have been reported to exist in equilibrium structures in aqueous solutions. Bajusz, S., et al., J. Med. Chem., 33: 1729 (1990). These structures, as shown below, include the arginine aldehyde, A, aldehyde hydrate, B, and two amino cyclol forms, C and D. The R group would represent the remainder of a given compound embodied in the present invention. The peptide aldehydes of the present invention include within their definition all the equilibrium forms.



Among other factors, the present invention is based on our finding that the novel compounds of our invention are active as inhibitors of urokinase. Compounds of the present invention exhibit activity in inhibiting angiogenesis.

In another aspect, the present invention is directed to pharmaceutical compositions comprising a therapeutically effective amount of a compound of the present invention and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention is directed to methods of using the compounds and pharmaceutical compositions of the present invention for inhibition of urokinase.

Definitions

In accordance with the present invention and as used herein, the following terms are defined to have following meanings, unless explicitly stated otherwise:

The term "alkenyl" refers to unsaturated aliphatic groups having at least one double bond.

The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and cyclic (including polycyclic) groups.

The terms "alkoxy" and "alkoxyl" refer to a group having the formula, R-O-, wherein R is an alkyl group.

The term "alkoxycarbonyl" refers to -C(O) OR wherein R is alkyl.

5 The term "aralkenyl" refers to an alkenyl group substituted with an aryl group. Preferably the alkenyl group has from 2 to about 6 carbon atoms.

 The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl, phenethyl, and the like, all of which may be optionally substituted. Preferably the alkyl group has from 1 to about 6 carbon atoms.

 The term "aryl" refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes a carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

 The term "aryloxy" refers to a group having the formula, R-O-, wherein R is an aryl group.

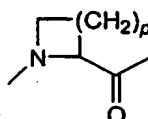
 The term "aralkoxy" refers to a group having the formula, R-O-, wherein R is an aralkyl group.

 The term "amino acid" refers to both natural, unnatural amino acids in their D and L stereo isomers if their structures allow such stereoisomeric forms, and their analogs. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid,

2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4-diaminoisobutyric acid, demosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically modified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The term "amino acid residue" refers to radicals having the structure: (1) $-C(O)-R-NH-$, wherein R typically is $-CH(R')$, wherein R' is H or a carbon containing substituent;

or (2)  , wherein p is 1, 2 or 3 representing the

azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

"Biaryl" refers to phenyl substituted by carbocyclic or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring.

5 "Brine" refers to an aqueous saturated solution of sodium chloride.

"Carbocyclic aryl" refers to aromatic groups wherein the ring atoms on the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and naphthyl groups, all of which may be optionally substituted. Suitable carbocyclic aryl groups include phenyl and naphthyl. Suitable substituted carbocyclic aryl groups include indene and phenyl substituted by one to two substituents such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkoxy carbonyl, halogen, trifluoromethyl, difluoromethyl, nitro, and cyano. Substituted naphthyl refers to naphthyl, more preferably 1- or 2-naphthyl, substituted by Y_1 , Y_2 and/or Y_3 as defined in connection with formula (I) hereinabove.

"Cycloalkenyl" refers to a cyclic alkenyl group. 20 Suitable cycloalkenyl groups include, for example, cyclopentenyl and cyclohexenyl.

"Cycloalkyl" refers to a cyclic alkyl group having at least one ring and includes polycyclic groups, including fused ring cyclic alkyl groups. Suitable cycloalkyl groups include, for example, cyclohexyl, cyclopropyl, cyclopentyl, and cycloheptyl. 25

"Cyclohexylmethyl" refers to a cyclohexyl group attached to CH_2 .

"Fused carbocyclic" refers to a multicyclic fused carbocyclic ring having both aromatic and non-aromatic rings. Suitable fused carbocyclic rings include fluorenyl, tetralin and the like. 30

"Fused carbocyclic alkyl" refers to an alkyl group substituted with a fused carbocyclic ring moiety, preferably a multicyclic fused carbocyclic ring including both aromatic and non-aromatic rings. Suitable fused carbocyclic alkyl groups include fluorenylmethyl, and the like.

The term "halogen" refers to fluorine, chlorine, bromine and iodine.

"Heteroaralkenyl" refers to an alkenyl group substituted with a heteroaryl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkenyl group has from 2 to about 6 carbon atoms.

"Heteroaralkyl" refers to an alkyl group substituted with a heteroaryl, such as picolyl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkyl group has from 1 to about 6 carbon atoms.

"Heteroaryl" refers to aromatic groups having from 1 to 14 carbon atoms and the remainder of the ring atoms are heteroatoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Suitable heteroatoms include oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, and suitable heterocyclic aryls

include furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, imidazolyl, and the like.

"Heterocyclo" refers to a reduced heterocyclic ring system comprised of carbon, nitrogen, oxygen and/or sulfur atoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

10 "Heterocycloalkyl" refers to an alkyl group substituted with a heterocyclo group, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for
15 Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkyl group has from about 1 to about 6 carbon atoms.

The term "lower" referred to herein in connection with organic radicals or groups defines such radicals or groups
20 with one and up to and including 5 carbon atoms, preferably up to and including 4 carbon atoms, and advantageously one or two carbon atoms. Such radicals or groups may be straight chain or branched chain.

"Perfluoroalkyl" refers to an alkyl group which has
25 every hydrogen replaced with fluorine.

"Perfluoroaryl" refers to an aryl group which has every hydrogen replaced with fluorine.

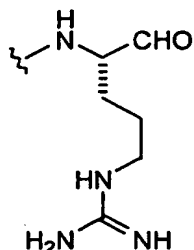
"Perfluoroarylalkyl" refers to an aralkyl group in which every hydrogen on the aryl moiety is replaced with fluorine.

30 "Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic

acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present

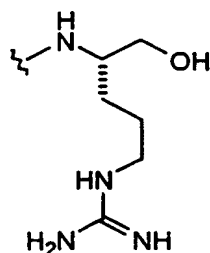
5 invention.

The term "Arg-al" refers to the residue of L-argininal which has the formula:

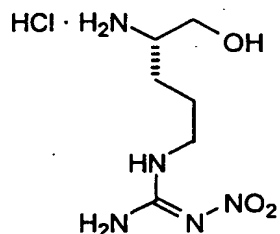


The term "Arg-ol" refers to the residue of L-argininol which has the formula:

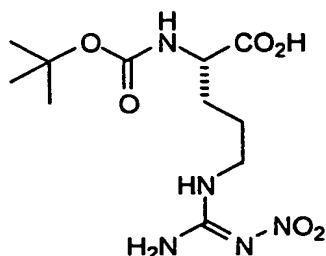
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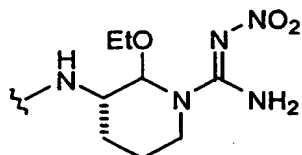
"(S)-N⁹-nitroargininol hydrochloride" refers to the compound which has the formula:



15 The term "N- -t-butoxycarbonyl-N⁹-nitro-L-arginine" refers to the compound which has the formula:



The term "L-N⁹-nitroarginal ethyl cyclol" refers to a group having the formula:



5 See also U.S. Patent No. 5,514,777.

"Bn" refers to benzyl.

"Boc" refers to t-butoxycarbonyl.

"BzISO₂" refers to benzylsulfonyl.

"Cbz" or "CBz" refers to benzyloxycarbonyl.

10 "DCA" refers to dichloroacetic acid.

"DCC" refers to N,N'-dicyclohexylcarbodiimide.

"DCM" refers to dichloromethane.

"DMF" refers to N,N-dimethylformamide.

"DMSO" refers to dimethyl sulfoxide.

15 "DMAP" refers to 4-N,N-dimethylaminopyridine.

"EDC" refers to 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride salt.

"Et₃N" refers to triethylamine.

"EtOH" refers to ethanol.

20 "HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

"HCl" refers to hydrochloric acid.

"HOAc" refers to acetic acid

"HPLC" refers to high pressure liquid chromatography.

25 "HOBt" refers to 1-hydroxybenzotriazole monohydrate.

"i-BuOCOCl" refers to isobutylchloroformate.

"LiAlH₄" refers to lithium aluminum hydride.

"LiAlH₂(OEt)₂" refers to lithium aluminum hydride diethoxide.

5 "Me" refers to methyl.

"NMM" refers to N-methylmorpholine.

"PhB(OH)₂" refers to phenylboronic acid.

"PyBOP" refers to benzotriazole-ly-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.

10 "TFA" refers to trifluoroacetic acid.

"THF" refers to tetrahydrofuran.

"TLC" refers to thin layer chromatography.

Brief Description Of The Drawings

Figure 1 depicts a reaction scheme for synthesis of an
15 intermediate useful in the solid phase synthesis of compounds
of the present invention having an argininal at P1. In this
figure, "i" through "vii" are defined as: i) 4-
methylmorpholine, EDC, and 1-hydroxybenzotriazole in
anhydrous acetonitrile; stirring at room temperature 16
20 hours, 78% recovery after workup; (ii) lithium aluminum
hydride/tetrahydrofuran at -78°C under N₂ one hour; warming
to room temperature, cooling to -78°C and quench with sodium
bisulfate, yield 76% after workup; iii) ethyl 6-
hydroxyhexanoate in acetonitrile, aqueous HCl; capping excess
25 ethyl 6-hydroxyhexanoate with acetic anhydride and pyridine,
93.7% yield after workup; iv) ethanol/water/acetic acid
(4:1:1), 10% palladium on carbon, H₂ at 40 psi for 16 hours,
94.4% yield after workup; v) DCM, 1 N NaOH, pH 11-13,
allylchloroformate, 80% yield after workup; vi) ethanol, 3N
30 LiOH, 1N HCl to pH 2-3, 83% yield after workup; and vii)
amino methylated polystyrene resin ("AM resin"), PyBOP in

DMF, diisopropylethylamine, coupling determined by Kaiser test; capping with DMF/acetic acid/triethylamine (8:1:1), 92% yield after workup. See also Examples 1 to 7.

Figure 2 depicts a reaction scheme for the solid phase synthesis of a compound of the present invention using resin 1-9 (See Figure 1). In this figure, "i" through "viii" are defined as i) DCM, TFA, and thioanisole; ii) Fmoc-alanine, 1-hydroxybenzotriazole, TBTU, and diisopropylethylamine in DMF, 99.5% coupling efficiency; iii) 50% piperidine in DMF; iv) 10 DMF, N- α -Fmoc-D-serine (O-t-butyl), 1-hydroxybenzotriazole, TBTU, diisopropylethylamine; v) 50% piperidine in DMF; vi) isobutylchloroformate in DMF, diisopropylethylamine; vii) methylsulfoxide, tetrahydrofuran, 1 N HCl, morpholine, tetrakis triphenylphosphine palladium; and viii) 15 TFA/DCM/H₂O (6:3:1), purification by semi-preparative reverse phase HPLC. See also Example 8.

Figure 3 depicts a reaction scheme for solution phase synthesis of a compound of the present reaction having an argininal at P1 using a L-N⁹-nitroargininal ethyl cyclol 20 intermediate. Compound 3-1 is N- α -Cbz-D-serine (O-t-butyl), compound 3-2 is alanine methyl ester, hydrochloride salt. In this figure, "i" through "vii" are defined as i) EDC, 1-hydroxybenzotriazole and acetonitrile, diisopropylethylamine to give Cbz-D-Ser (O-t-butyl)-Ala-OMe; (ii) ethanol/acetic 25 acid/water (4:1:1), 10% Pd on carbon, 45 psi H₂ for 2 hours, 95% yield after work-up; iii) acetonitrile, benzenesulfonyl chloride, diisopropylethylamine, 43% yield after work-up; iv) methanol, 1.0 M lithium hydroxide, acidification on DOWEX ion exchange resin, eluting with methanol/water, 95% yield after 30 work-up; v) 1-hydroxybenzotriazole, acetonitrile, diisopropylethylamine, 95% yield after work-up; vi) hydrogenation over 10% Pd on carbon in ethanol/acetic

acid/water (4:1:1) at 50 psi, isolation of benzylsufonyl-D-Ser(O-t-Bu)-L-Ala-L-argininal ethyl cyclol; and vii) 6 M HCl with stirring, 6.5M ammonium acetate to pH 4, purification by preparative reverse-phase HPLC, 15% yield for three (v-vii) steps. See also Examples 17 to 20.

Figure 4 depicts a reaction scheme for the synthesis of compounds of the present invention having an arginine ketoamide group at P1. In this figure "i" through "ix" are defined as: i) phenethylamine, BOP and 1-hydroxybenzotriazole, DMF, 4-methylmorpholine, 89% yield after workup; ii) HCl in ethyl acetate, to give N⁹-nitroArg-COH-phenethylamide hydrochloride salt; iii) t-butoxycarbonyl-Ala-OH, EDC, 1-hydroxybenzotriazole, and acetonitrile, diisopropylethylamine, 95% yield after workup; iv) HCl in ethyl acetate, 98% yield after workup; v) t-butoxycarbonylserine benzyl ether EDC and 1-hydroxybenzotriazole, acetonitrile, diisopropylethylamine, yield 92% after workup; vi) HCl in ethyl acetate, 98% yield after workup; vii) acetonitrile, isobutyl chloroformate, diisopropylethylamine, 85% yield after workup; viii) EDC, methylsulfoxide and dichloroacetic acid, 68% yield after workup; ix) anisole, HF at -20°C, 26% yield after workup and purification by preparative reverse phase HPLC. See also Examples 27 to 34.

Figure 5 depicts a reaction scheme for an alternate solution phase sythetic route to prepare Compound 1 (the compound depicted in Figure 2) an L-N⁹-nitroargininal ethyl cyclol intermediate. In this figure, "i" through "vi" are defined as: i) isobutyl chloroformate, sodium carbonate, water, 99.5% yield after workup; ii) alanine t-butyl ester, hydrochloride salt, EDC, and hydroxybenzotriazole in acetonitrile; diisopropylethylamine, quantitative yield after

workup; iii) TFA, DCM, quantitative yield after workup; iv) N⁹-nitroargininal ethyl cyclol, HCl salt, EDC, and hydroxybenzotriazole in acetonitrile; diisopropylethylamine, 50% yield after workup; v) ethanol/acetic acid/water (4:1:1), 10% palladium on carbon, H₂ at 50 psi, 4 hours; vi) 3.0M HCl; preparative reverse phase HPLC; 62% yield of compound 1 for two (v and vi) steps. See also Examples 54 to 59.

Figure 6 depicts a reaction scheme for the synthesis of a protected argininal hydrazonylcarbonyl aminomethylated polystyrene resin used in the solid phase synthesis of compounds of the present invention having an argininal at P1. Compound 6-1 is N- α -fluorenylmethyloxycarbonyl- ω , ω -di-N-t-butoxycarbonyl-arginine. In this figure, "i" through "iv" are defined as: i) acetonitrile, potassium carbonate, methyl iodide, 50°C, ethyl acetate; ii) THF and methanol, calcium chloride, ice bath, sodium borohydride, stirring, 71% yield after workup; iii) dimethylsulfoxide, and toluene, ice bath, EDC and dichloroacetic acid; and iv) DCM, HCAM resin, ambient temperature 16 to 24 hours. See also Examples 41 to 45.

Figure 7 depicts a reaction scheme for solid phase synthesis of compounds of the present invention having an argininal at P1 and using the intermediate of Figure 6. In this figure, "i" through "ix" are defined as: i) 30% piperidine/DMF; ii) N- α -Fmoc-azetidine-2-carboxylic acid, 1-hydroxybenzotriazole, TBTU, and diisopropylethylamine in DMF; iii) 30% piperidine/DMF; iv) N- α -Fmoc-D-serine-t-butyl ether, 1-hydroxybenzotriazole, TBTU, and diisopropylethylamine in DMF; v) double coupling: N- α -Fmoc-serine-o-t-butyl ether, 1-hydroxybenzotriazole, TBTU and diisopropylethylamine in DMF, 97% coupling efficiency after work up; vi) 30%

piperidine/DMF; vii) benzenesulfonyl chloride and diisopropylethylamine in DMF; viii) double coupling: benzenesulfonyl chloride and diisopropylethylamine in DMF, 96% coupling efficiency after workup; and ix) TFA/water
5 (9:1), 26% yield after purification by semipreparative reverse phase HPLC. See also Examples 46 to 53.

Figure 8 depicts a reaction scheme for preparation of an intermediate used for the synthesis of compounds of the present invention having a 3-piperidinyl-(N-guanidino)
10 alaninal at P1. In this figure, "i" through "vi" are defined as: i) thionyl chloride, methanol; ii) di-tert-butyl dicarbonate, pH 7 to 8; iii) hydrogen gas, platinum oxide in ethanol, water and acetic acid; iv) bis-benzyloxycarbonyl S-methylisothiurea, base, tetrahydrofuran; v) calcium
15 chloride, sodium borohydride in tetrahydrofuran and ethanol; vi) HCl, ethyl acetate. "*" indicates the position of an asymmetric carbon atom. See also Examples 60 to 64.

Figure 9 depicts a reaction scheme for preparation of an intermediate used for the synthesis of compounds of the present invention having a 3-amidinophenylalaninal at P1. In
20 this figure, "i" through "xi" are defined as i) potassium iodide, dioxane; 2.5 M sodium ethoxide in ethanol, argon atmosphere, reflux 6 hours; yield after work up 60%; ii) pyridine, triethylamine; H₂S(g), stirred at room temperature
25 16 hours, yield after work up 98%; iii) acetone, iodomethane, reflux 30 minutes, filtration, methanol; iv) ammonium acetate, reflux 1 hour, filter and dry; v) concentrated HCl, reflux 3 hours, yield after workup 30%; vi) dioxane, sodium bicarbonate, di-t-butyl dicarbonate, stir 18 hours at room
30 temperature; vii) 4°C, 4.0 N NaOH to pH 12; 4-methoxy-2,3,6-trimethylbenzene sulfonyl chloride in dioxane; 1.0 N HCl to pH 7 to 8, water, yield after work up 68%, viii) O, N-

dimethylhydroxylamine hydrochloride, hydroxybenzotriazole hydrate, 4-methylmorpholine, THF, stir 2 hours, yield after workup 69%; ix) LiAlH_4 , THF, -78°C ; aqueous potassium bisulfate, yield after workup 86%; x) 4-

- 5 benzhydrylsemicarbizide trifluoroacetate salt (the compound of Example 65), sodium acetate trihydrate in ethanol, reflux, yield after workup 89%; and xi) 50% TFA/DCM, add to ether, yield after workup 79%. See also Examples 65 to 72.

Figure 10 depicts a reaction scheme for the preparation
10 of an intermediate which is used in the synthesis of compounds of the present invention having a 4-piperidinyl-(N-guanidino)alaninal at P1. In this figure, "i" through "vi" are defined as i) thionyl chloride, methanol; ii) di-tert-butyl carbonate, pH 7 to 8; iii) hydrogen gas, platinum oxide
15 in ethanol, water and acetic acid; iv) bis-benzyloxycarbonyl S-methylisothiurea, base, tetrahydrofuran; v) calcium chloride, sodium borohydride in tetrahydrofuran and ethanol; and vi) HCl in ethyl acetate. "*" indicates the position of an asymmetric carbon atom. See Example 73.

20 Figure 11 depicts a reaction scheme for synthesis of a compound of the present invention having a 3-guanidino-piperidinal group at P1 using an intermediate such as 8-6 of Figure 8. In this figure, "i" through "ix" are defined as:
i) isobutylchloroformate, sodium bicarbonate, aqueous
25 dioxane; ii) alanine t-butyl ester, EDC, HOBT, NMM; iii) H_2 , Pd/C; iv) pyridine, phenyl chloroformate; v) TFA/DCM; vi) EDC, HOBT, NMM; vii) H_2 , Pd/C; viii) EDC, DCA, DMSO, toluene; and ix) H_2O , pH 7; preparative reverse-phase HPLC. See
Example 74.

30 Figure 12 depicts a reaction scheme for synthesis of a compound of the present invention having an amidino-

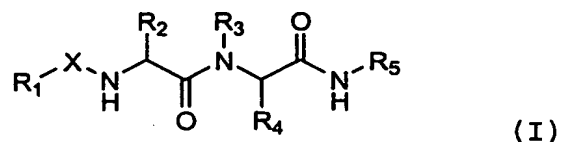
phenylalaninal at P1 using an intermediate such as 9-10 of Figure 9. In this figure, "i" through "iii" are defined as: i) EDC, HOBT, NMM; ii) HF, anisole; and iii) 90% aqueous TFA; preparative reverse-phase HPLC. See Example 75.

- 5 Figure 13 depicts a reaction scheme for the synthesis of a compound of the present invention where R_2 is $-\text{CH}_2\text{OA}_2$ and A_2 is $-\text{C}(=\text{O})\text{R}_9$, using intermediate 13-1 (5-5 of Figure 5 (Example 57)). In this figure "i" through "iii" are defined as: i) pyridine, R_9COCl ; ii) H_2 , 10% Pd/C, EtOH, H_2O ; iii) 10 aqueous HPF_6 , acetonitrile.

Detailed Description Of The Invention

1. Preferred Compounds

Compounds of the present invention have the formula:



15 wherein:

(a) X is selected from the group consisting of $-\text{S}(\text{O})_2-$, $-\text{N}(\text{R}')-\text{S}(\text{O})_2-$, $-(\text{C}=\text{O})-$, $-\text{OC}(=\text{O})-$, $-\text{NH}-\text{C}(=\text{O})-$, $-\text{P}(\text{O})(\text{R}')-$, and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with 20 the proviso that when X is $-\text{P}(\text{O})(\text{R}')-$, then R' is not hydrogen;

(b) R_1 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms which is 25 optionally substituted with Y_1 ,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon atoms which is optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2,

including, $-\text{N} \begin{smallmatrix} \text{---} \end{smallmatrix} \text{V}$ wherein $-\text{N} \begin{smallmatrix} \text{---} \end{smallmatrix} \text{V}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}(=\text{O})-$, $-\text{S}(\text{O})_2-$ or $-\text{S}-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,

(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

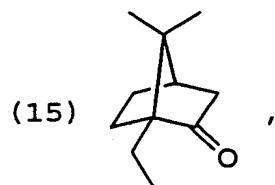
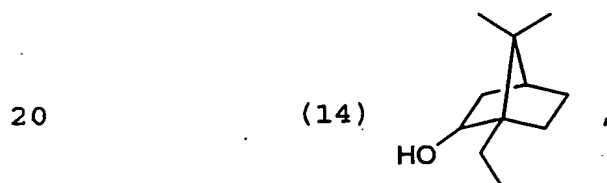
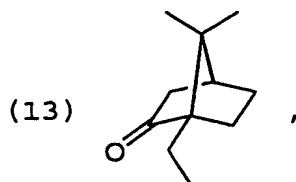
(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

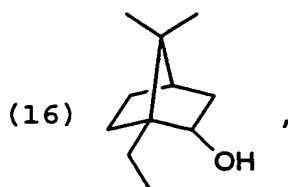
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

5 (10) heteroaralkyl of about 5 to 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

10 (11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(12) heteroaralkenyl of about 5 to 14 ring atoms with the ring atoms selected from carbon and heteroatoms, 15 wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,





(17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms;

(18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

(19) perfluoroaryl of about 6 to about 14 carbon atoms,

(20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(21) hydrogen when X is a direct link;

wherein each Y_1 , Y_2 , and Y_3 is independently selected and is

(i) selected from the group consisting of halogen, cyano, nitro, tetrazolyl, guanidino, amidino, methylguanidino, $-\text{CF}_3$, $-\text{CF}_2\text{CF}_3$, $-\text{CH}(\text{CF}_3)_2$, $-\text{C}(\text{OH})(\text{CF}_3)_2$, $-\text{OCF}_3$, $-\text{OCF}_2\text{H}$, $-\text{OCF}_2\text{CF}_3$, $-\text{OC}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{NHZ}_1$, $-\text{OC}(\text{O})\text{NZ}_1\text{Z}_2$, $-\text{NHC}(\text{O})\text{Z}_1$, $-\text{NHC}(\text{O})\text{NH}_2$, $-\text{NHC}(\text{O})\text{NZ}_1$, $-\text{NHC}(\text{O})\text{NZ}_1\text{Z}_2$, $-\text{C}(\text{O})\text{OH}$, $-\text{C}(\text{O})\text{OZ}_1$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{NHZ}_1$, $-\text{C}(\text{O})\text{NZ}_1\text{Z}_2$, $-\text{P}(\text{O})_3\text{H}_2$, $-\text{P}(\text{O})_3(\text{Z}_1)_2$, $-\text{S}(\text{O})_3\text{H}$, $-\text{S}(\text{O})_m\text{Z}_1$, $-\text{Z}_1$, $-\text{OZ}_1$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NHZ}_1$, $-\text{NZ}_1\text{Z}_2$, $-\text{N-morpholino}$, $-\text{S}(\text{CF}_2)_q\text{CF}_3$, and $-\text{S}(\text{O})_m(\text{CF}_2)_q\text{CF}_3$,

wherein m is 0, 1 or 2, q is an integer from 0 to 5, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms, or

(ii) Y_1 and Y_2 are selected together to be $-\text{O}[\text{C}(\text{Z}_3)(\text{Z}_4)]_r\text{O}-$, wherein r is an integer from 1 to 4 and Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of

about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms;

(c) R_2 is selected from the group consisting of H,
5 -CH₃, -C₂H₅, -(CH₂)₂OH, -(CH₂)₂OA₂, -CH(R₆)OH, -CH(R₆)OA₂ and
-CH₂NH-X'-R₆ wherein A₂ is -C(=O)OR₉ or -C(=O)R₉; X' is
selected from the group consisting of -S(O)₂-, -S(O)₂-N(R'')-,
-(C=O)-, -C(=O)-O-, -C(=O)-NH-, -P(O)(R'')-, and a direct
link, wherein R'' is hydrogen, alkyl of 1 to about 4 carbon
10 atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of
about 7 to about 16 carbon atoms with the proviso that when
X' is -P(O)(R'')-, then R'' is not hydrogen; R₆ is selected
from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms,
15 optionally substituted with Y₁,

(2) alkyl of 1 to about 3 carbon atoms substituted
with cycloalkyl of about 5 to about 8 carbon atoms, which is
optionally mono-, di-, or tri-substituted on the ring with
Y₁, Y₂, and/or Y₃,

20 (3) cycloalkyl of 3 to about 15 carbon atoms,
which is optionally mono-, di-, or trisubstituted on the ring
with Y₁, Y₂, and/or Y₃,

(4) heterocycloalkyl of 4 to about 10 ring atoms
with the ring atoms selected from carbon and heteroatoms,
25 wherein the heteroatoms are selected from the group
consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1
or 2, which is optionally mono-, di-, or tri-substituted on
the ring with Y₁, Y₂, and/or Y₃,

(5) heterocyclo of 4 to about 10 ring atoms with
30 the ring atoms selected from carbon and heteroatoms, wherein
the heteroatoms are selected from the group consisting of
oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2,

including $-\text{N} \begin{smallmatrix} \circ \\ \text{V} \end{smallmatrix}$, wherein $-\text{N} \begin{smallmatrix} \circ \\ \text{V} \end{smallmatrix}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}(=\text{O})-$, $-\text{S}(\text{O})_2-$ or $-\text{S}-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,

5 (6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(7) aryl of about 6 to about 14 carbon atoms which
10 is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen,
15 and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(9) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

20 (10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and
25 optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

30 (12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and

heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 , and

5 (13) hydrogen; and

R_9 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms, optionally substituted with Y_1 ,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including $-\text{N} \begin{smallmatrix} \circlearrowleft \end{smallmatrix} \text{V}$, wherein $-\text{N} \begin{smallmatrix} \circlearrowleft \end{smallmatrix} \text{V}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}(=\text{O})-$, $-\text{S}(\text{O})_2-$ or $-\text{S}-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,

(6) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

5 (7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

10 (8) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from
15 oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 , and

(10) hydrogen with the proviso that R_9 is not
20 hydrogen when A_2 is $-C(=O)OR_9$;

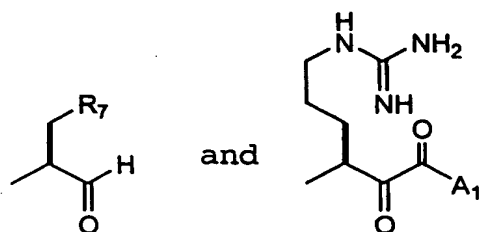
(d) R_3 is selected from H or methyl, or R_3 and R_4 are selected together as set forth in (f);

(e) R_4 is in the S configuration and is selected from the group consisting of H, $-CH_2-S-CH_3$, $-CH_2OH$, $-CH_2CN$, lower
25 alkyl of 1 to about 3 carbon atoms, $-CH_2C\equiv CH$, $-CH_2CH=CH_2$ and $-CH=CH_2$ or R_3 and R_4 are selected together as set forth in (f);

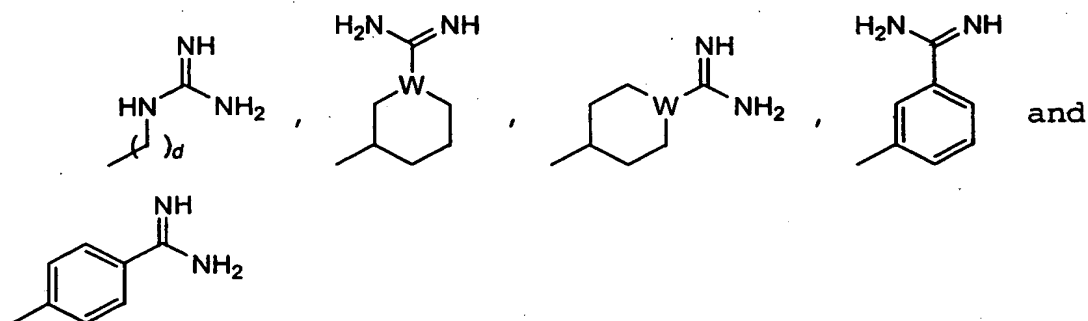
(f) alternatively, R_3 and R_4 are selected together to be in the S configuration to give a group at P2 selected from
30 the group consisting of prolyl, pipecholy, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, and 3,4-dehydroprolyl;

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(g) R_5 is selected from the group consisting of



wherein R_7 is selected from



wherein d is an integer from 1 to 3 and W is $-\text{N}-$ or $-\text{CH}-$; and

(h) A_1 is $-\text{NHR}_8$, wherein R_8 is alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 15 carbon atoms, all optionally mono-, di or tri-substituted with Y_1 , Y_2 and/or Y_3 or is hydrogen; and pharmaceutically acceptable salts thereof.

Preferred X groups include $-\text{S}(\text{O})_2-$, $-\text{OC}(=\text{O})-$, $-\text{NH}-\text{C}(=\text{O})-$, and a direct link. Especially preferred are $-\text{S}(\text{O})_2-$ and $-\text{OC}(=\text{O})-$.

Preferred R_1 groups include alkyls, especially isobutyl, 2-ethylhexyl, methyl, butyl, isopropyl, cyclohexylmethyl, and cyclohexylpropyl; cycloalkyl, especially $(-)$ menthyl, $(+)$ menthyl, and cyclohexyl; aryls, especially naphthyl and phenyl; aralkyls, especially benzyl, 3-phenylpropyl, and 2-phenylethyl; and fused carbocyclic alkyls, especially fluorenylmethyl. Especially preferred R_1 groups include phenyl, benzyl, 2-phenylethyl, isobutyl, and 3-phenylpropyl.

Preferred combinations of R_1 -X- include phenyl-S(O)₂-, benzyl-S(O)₂-, 2-phenylethyl-S(O)₂-, 3-phenylpropyl-S(O)₂-, benzyl-OC(=O)-, and isobutyl-OC(=O)-.

Preferred R_2 groups include H, -CH₃, -C₂H₅, -CH₂NH-X'-R₆ and -CH(R₆)OH, wherein R₆ is hydrogen, alkyl, especially methyl, or aralkyl. Preferred chirality at the alpha carbon is R. When chiral, preferred chirality at the beta carbon is R. Preferred R_2 groups are those that define the P₃ position as glycine, d-seryl (-CH(R₆)OH where R₆ is H), (R,R)d-allothreonyl (-CH(R₆)OH where R₆ is methyl), d-2-aminobutyryl, N-β-methyloxycarbonyl-d-2,3-diaminopropionyl (-CH₂NH-X'-R₆ where R₆ is CH₃ and X' is -(C=O)O-), N-β-(2-phenylethylcarbonyl)-d-2,3-diaminopropionyl (-CH₂NH-X-R₆ where R₆ is 2-phenylethyl and X' is -(C=O)-), and N-β-benzyloxycarbonyl-d-2,3-diaminopropionyl (-CH₂NH-X'-R₆ where R₆ is benzyl and X' is -(C=O)O-). Especially preferred R_2 groups are those which define P₃ as d-seryl (R₆ is H) or (R,R)d-allothreonyl (R₆ is methyl).

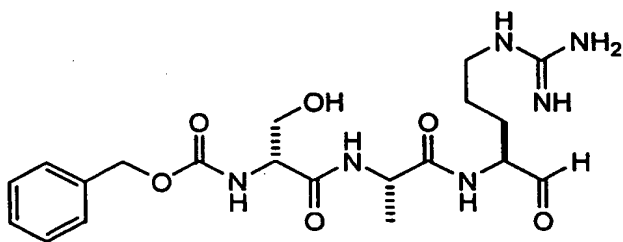
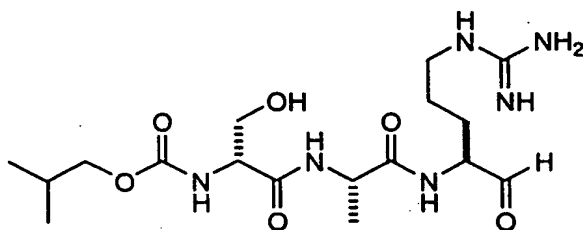
Alternate preferred R_2 groups include -(CH₂)₂OA₂ and -CH(R₆)OA₂, more preferably -CH(R₆)OA₂; preferably R₆ is H. More preferably R_2 is selected so that P₃ is defined as an acyl or carbonate ester of d-seryl. Compounds wherein R_2 is -(CH₂)₂OA₂ or -CH(R₆)OA₂ may act as prodrugs.

A preferred R_3 group, when R_3 and R_4 are not selected together, is hydrogen. A preferred R_4 group, when R_3 and R_4 are not selected together, is methyl or propargyl. When R_3 and R_4 are selected together, prolyl, 4-cis-hydroxyprolyl, 3,4-dehydroprolyl, and azetidine-2-carbonyl- are preferred selections to define a group at P₂.

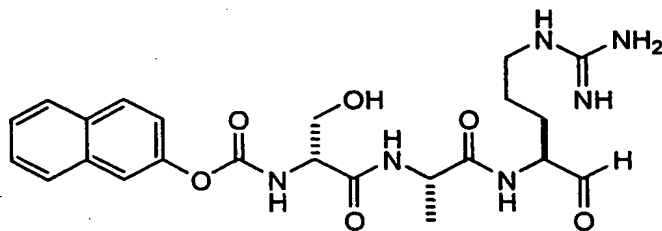
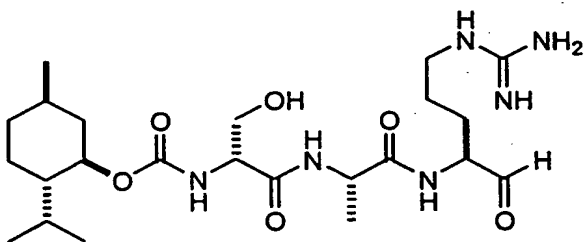
The preferred R_5 group is -CH((CH₂)₃NHC(=NH)NH₂)CHO, to give an arginine aldehyde at P₁.

Among the claimed compounds, those having an R₂ element that defines d-serine or d-allothreonine at that position of the compound and an arginine aldehyde at R₅ are preferred. Especially preferred are such compounds also having either i) a hydrogen at R₃ and methyl at R₄ (P2 is alanine), or ii) having R₃ and R₄ selected together so that P2 is prolyl, azetidine-2-carbonyl, or 3,4-dehydroprolyl.

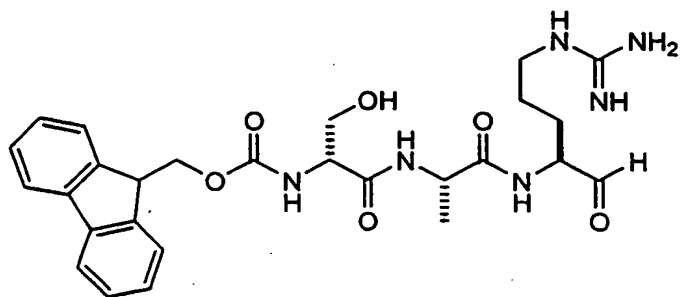
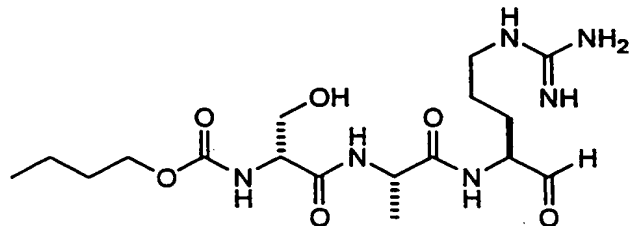
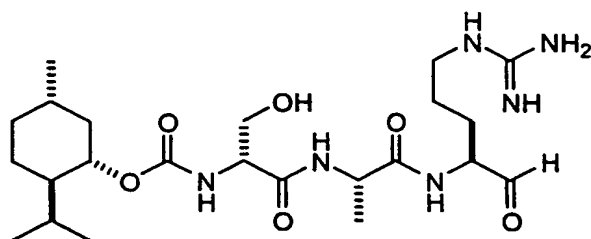
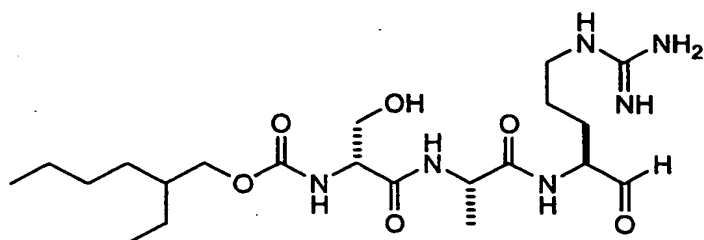
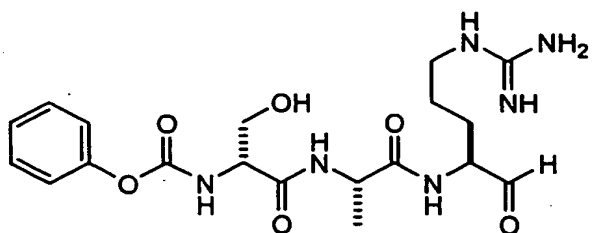
Preferred compounds of the present invention include:



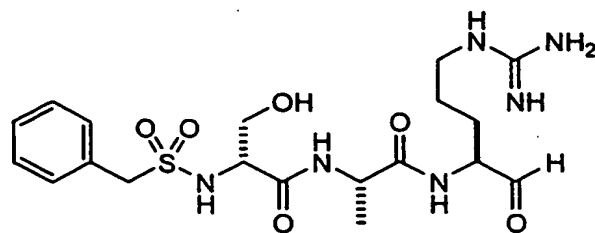
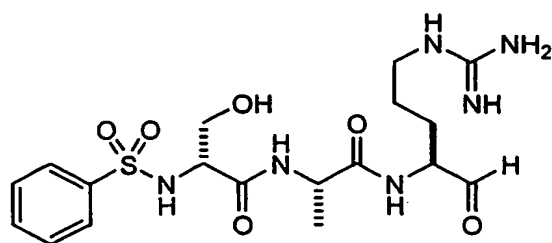
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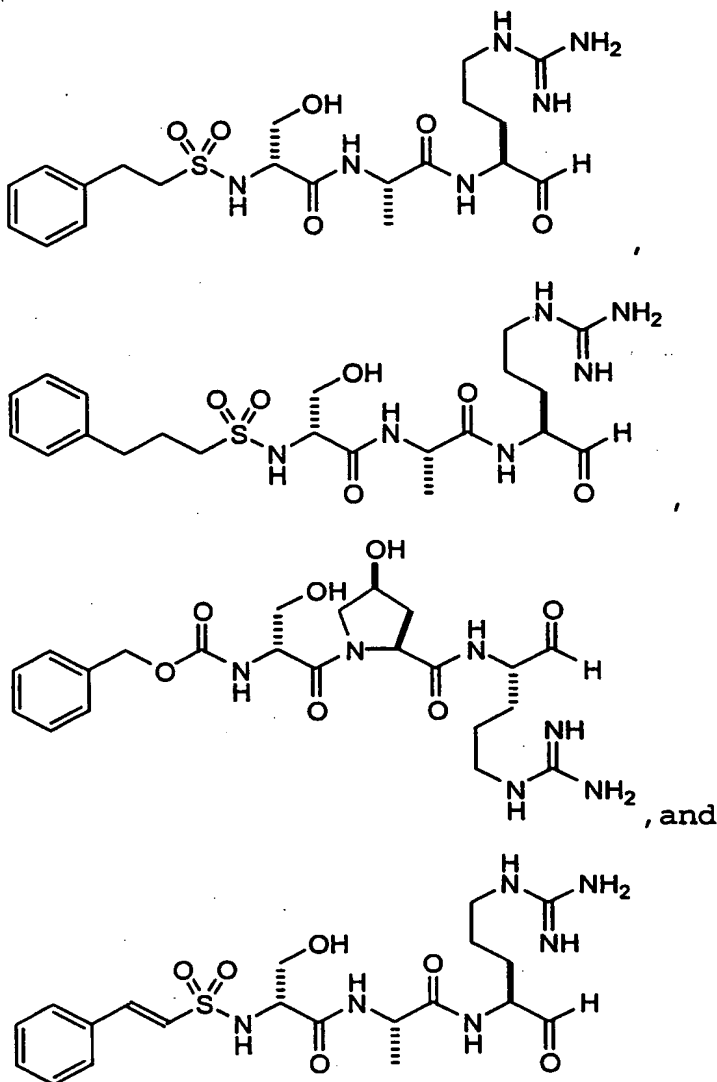
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2. Preparation of Preferred Compounds

Figures 1 to 12 depict synthetic schemes for preparation of certain preferred compounds of the present invention.

Figure 1 depicts synthesis of N- α -t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-amino-methylated polysyrene resin) cyclol which is useful in solid phase synthesis of compounds of the present invention having an arginine aldehyde at P1. This synthesis is described in detail by Examples 1 to 7.

Figure 2 depicts solid phase synthesis of a compound of the present invention using the resin depicted in Figure 7. This synthesis is described further in Example 8.

Figure 3 depicts solution phase synthesis of compounds of the present invention using a L-N⁹-nitroargininal ethyl cyclol intermediate. See also Examples 17 to 21 and 22 to 26.

Figure 4 depicts a synthesis scheme for compounds of the present invention having an arginine ketoamide group at P1. Synthesis of such a compound is further described by Examples 27 to 34.

Examples 35 to 40 describe solution phase synthesis of a preferred compound of the present invention using an L-N⁹-nitroargininal ethyl cyclol intermediate.

Figure 5 depicts an alternate synthetic route to prepare the compound depicted in Figure 2 using solution phase synthesis and an L-N⁹-nitroargininal ethyl cyclol intermediate. See also Examples 54 to 59.

Figure 6 depicts a synthesis scheme and Examples 41 to 45 further describe synthesis of argininal-hydrazonylcarbonyl aminomethylated resins which may be used to prepare compounds of the present invention.

Figure 7 depicts a synthetic scheme for solid phase preparation of compounds of the present invention using the resin of Example 45. Examples 46 to 53 further describe such solid phase syntheses.

Figure 8 depicts a synthetic scheme for preparation of an intermediate used in synthesizing compounds of the present invention having an 3-piperidinyl-(N-guanidino)-alaninal group at P1. Examples 60 to 64 describe its preparation in further detail.

Figure 9 depicts a reaction scheme for preparation of an intermediate which is used in the synthesis of compounds of the present invention having a 3-amidinophenylalaninal group at P1. Intermediates used in the preparation of compounds of formula (I) having a 4-amidinophenylalaninal group at P1 may be prepared according to the reaction scheme depicted in Figure 9 and described in Examples 65 to 72, using the appropriate α -bromo-para-tolunitile starting material.

Figure 10 depicts a reaction scheme for the preparation of an intermediate which is used in the synthesis of compounds of the present invention having a 4-piperidinyl-(N-guanidino)alaninal group at P1. This intermediate is prepared using procedures similar to those described in Examples 60 to 64 and using the appropriate starting materials.

Figure 11 depicts a reaction scheme for preparation of a compound of the present invention having a 3-piperidinyl-(N-guanidino)alaninal group at P1 using an intermediate such as 8-6 of Figure 8.

Figure 12 depicts a reaction scheme for preparation of a compound of the present invention having a 3-amidinophenylalaninal group at P1 using an intermediate such as 9-10 of Figure 9.

Figure 13 depicts a reaction scheme for the preparation of a compound of the present invention having an esterified hydroxyl at P3.

Preferred means of chemically coupling (as for example, amide bond function) include formation of a peptide bond by using conventional coupling reagents known in the art. See Bodanszky, N. Peptide Chemistry, pp. 55-73, Springer-Verlag, New York (1988) and references cited therein. The chemical coupling may be either by means of one-step or two-step

coupling. In one-step coupling, the two coupling partners are coupled directly. Preferred coupling reagents for one-step coupling include DCC with HOBT, EDC with HOBT, EDC with HOAt, HBTU or TBTU. In two-step coupling, an
5 activated ester or anhydride of the C-terminal carboxy group of one coupling partner is formed prior to its coupling to the other coupling partner.

For preparation of certain compounds having hydrogenation-sensitive substituent groups, it is preferred
10 to avoid the use of hydrogen gas with palladium on carbon. Another preferred method for preparing compounds of the present invention containing hydrogenation sensitive groups such as alkenyl or aryl moieties substituted with halogen, cyano, nitro, or -S-Z₁, is to use boron
15 tris(trifluoroacetate), B(OCOCF₃)₃, to cleave the N⁹-nitro of the arginine group. The reagent is prepared by the reaction of BBr₃ and CF₃COOH in dichloromethane at 0°C. The reagent is also commercially available. Generally, the N⁹-nitro compound is treated with boron tris(trifluoroacetate) in
20 trifluoroacetic acid at 0°C. See, e.g., Fieser, M. and Fieser, L. F., Reagents for Organic Synthesis, p. 46, John Wiley & Sons, New York (1974); Pless, J., and Bauer, W. Angew. Chem., Internat. Ed., 12, 147 (1973).

In addition, another preferred reagent for selective
25 nitro group cleavage is titanium trichloride. This reagent is commercially available. The N⁹ nitro compound is treated with titanium trichloride in aqueous methanol containing an ammonium acetate buffer followed by exposure of the reaction mixture to air or dimethyl sulfoxide. See, e.g., Freidinger,
30 R.M., Hirschmann, R., and Veber, D.F., J. Org. Chem., 43, 4800 (1978).

Another preferred method for synthesizing these compounds having an L-argininal moiety is to use the di-N-t-butoxycarbonyl protecting group for the L-argininal moiety for groups incompatible with hydrogenation with palladium on carbon. For example, α -N-benzyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonylarginine is dissolved in acetonitrile and treated with hydroxybenzotriazole and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl salt to form α -N-benzyloxycarbonyl-omega,omega'-di-N-t-butoxycarbonyl-L-arginine lactam. The lactam is reduced by treatment with LiAlH_4 in THF at -70°C to provide α -N-benzyloxycarbonyl-omega,omega;-di-N-t-butoxycarbonyl-L-argininal. This aldehyde is protected as the diethyl acetal by treatment with ethanol and HCl. The N-benzyloxycarbonyl protecting group is removed by treatment with hydrogen gas and palladium on carbon to give omega, omega'-di-N-t-butoxycarbonyl-L-argininal diethyl acetal, HCl salt. This protected L-argininal moiety can then be coupled to a desired carboxylic acid by treatment with N-hydroxybenzotriazole and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl salt. The diethyl acetal and the di-Boc protecting groups are removed by treatment with hexafluorophosphoric acid in acetonitrile at 0°C . The reaction mixture is quenched by addition of 2.5M aqueous sodium acetate until pH 4 is reached. The mixture is filtered through a 2 micron filter. Preparative HPLC using 0.1% CF_3COOH in 10-40% aqueous acetonitrile provides the trifluoroacetate salt of the desired substituted L-argininal compound.

Figure 13 depicts a reaction scheme for the synthesis of a compound of the present invention where R_2 is $-\text{CH}_2\text{OA}_2$ and A_2 is $-\text{C}(=\text{O})\text{R}_9$. An intermediate such as 13-1 (Example 57) is reacted with an acid chloride R_9COCl in the presence of a

base such as pyridine. Intermediate 13-2 is hydrogenated (H_2 , 10% Pd/C in ethanol:water:acetic acid) and then treated with aqueous HPF_6 and acetonitrile to remove protecting groups without hydrolysis of the ester at R_2 . Compounds
5 where R_2 is $-(CH_2)_2OA_2$ or $-CH(R_6)OA_2$ where A_2 is $-C(=O)R_9$ may conveniently be prepared by reacting an appropriate intermediate corresponding to 13-1 with the appropriate acid chloride derivative, R_9COCl , preferably in the presence of a base such as pyridine. In preparing compounds where R_5 is an
10 argininal group, the product is then hydrogenated and treated with aqueous HPF_6 and acetonitrile to yield the product argininal without hydrolysis of the ester at R_2 .

Compounds where R_2 is $-(CH_2)_2OA_2$ or $-CH(R_6)OA_2$ wherein A_2 is $-C(=O)OR_9$ may be conveniently prepared by treating a
15 corresponding compound where R_2 is $-(CH_2)_2OH$ or $-CH(R_6)OH$ with the appropriate chloroformate derivative. In the preparation of compounds where P_1 is argininal, it is preferred to cap with the carbonate group prior to deprotecting the arginine side chain. Accordingly, it is preferred to treat the
20 corresponding N^9 -nitroargininal-ethylcyclool intermediate with the chloroformate derivative. (See, e.g., Example 76). The product is then hydrogenated and treated under hydrolysis conditions to yield the product argininal. (See, e.g., Examples 77 to 78).

25 3. Selection of Preferred Compounds

According to one aspect of the present invention, preferred compounds of the present invention are selected for their potency and selectivity toward inhibition of serine proteases, especially urokinase. Such evaluations are
30 routinely performed *in vitro*, following procedures such as those set forth in Example A. As described therein, and as

generally known, a target serine protease and its substrate are combined under assay conditions permitting reaction of the protease with its substrate. The assay is performed in the absence of test compound, and in the presence of increasing concentrations of the test compound. The concentration of test compound at which 50% of the serine protease activity is inhibited by the test compound is the IC_{50} value (Inhibitory Concentration) or EC_{50} (Effective Concentration) value for that compound. Within a series or group of test compounds, those having lower IC_{50} or EC_{50} values are considered more potent inhibitors of the serine protease than those compounds having higher IC_{50} or EC_{50} values. The IC_{50} measurement is often used for more simplistic assays, whereas the EC_{50} is often used for more complicated assays, such as those employing cells.

Preferred compounds according to this aspect of the present invention have an IC_{50} value of 100nM or less as measured in an *in vitro* assay for inhibition of urokinase activity. Especially preferred compounds have an IC_{50} value of less than 30nM.

The test compounds also are evaluated for selectivity toward a serine protease. As described in the Examples, and as generally known, a test compound is assayed for its potency toward a panel of serine proteases and other enzymes and an IC_{50} value or EC_{50} value is determined for each test compound in each assay system. A compound that demonstrates a low IC_{50} value or EC_{50} value for the target enzyme, e.g., urokinase, and a higher IC_{50} value or EC_{50} value for other enzymes within the test panel (e.g., tissue plasminogen activator, thrombin, Factor Xa), is considered to be selective toward the target enzyme. Generally, a compound is deemed selective if its IC_{50} value or EC_{50} value in the target

enzyme assay is at least one order of magnitude less than the next smallest IC_{50} value or EC_{50} value measured in the selectivity panel of enzymes.

Preferred compounds of the present invention have an IC_{50} value of 100nM or less as measured in an *in vitro* assay for inhibition of urokinase activity. Especially preferred compounds have an IC_{50} value in the *in vitro* urokinase inhibition assay that is at least one order of magnitude smaller than the IC_{50} value measured in the *in vitro* tPA inhibition assay. Compounds having a selectivity ratio of IC_{50} tPA assay: IC_{50} urokinase assay of greater than 100 are especially preferred.

Compounds of the present invention also are evaluated for their activity *in vivo*. The type of assay chosen for evaluation of test compounds will depend on the pathological condition to be treated or prevented by use of the compound, as well as the route of administration to be evaluated for the test compound.

For instance, to evaluate the activity of a compound of the present invention to reduce tumor growth through inhibition of urokinase, the procedures described by Jankun et al. [Canc. Res. 57:559-563, 1997] to evaluate PAI-1 can be employed. Briefly, the ATCC cell lines DU145, which expresses a high level of uPA, and LnCaP, which does not express uPA, are injected into SCID mice. After tumors are established, the mice are given test compound according to a dosing regime determined from the compound's *in vitro* characteristics. The Jankun et al. compound was administered in water. Tumor volume measurements are taken twice a week for about five weeks. A compound is deemed active if an animal to which the compound was administered exhibited decreased tumor volume, as compared to animals receiving

appropriate control compounds. Furthermore, a comparison of a compound's effect in animals injected with DU145 cells versus LnCaP cells can indicate whether the compound's effect was due to inhibition of urokinase or otherwise.

5 Another *in vivo* experimental model designed to evaluate the effect of p-aminobenzamidine, a purported urokinase inhibitory compound, on reducing tumor volume is described by Billström et al. [Int. J. Cancer 61:542-547, 1995].

To evaluate the ability of a compound of the present
10 invention to reduce the occurrence of, or inhibit, metastasis, the procedures described by Kobayashi et al. [Int. J. Canc. 57:727-733d, 1994] can be employed. Briefly, a murine xenograft selected for high lung colonization potential is injected into C57B1/6 mice i.v. (experimental
15 metastasis) or s.c. into the abdominal wall (spontaneous metastasis). Various concentrations of the compound to be tested can be admixed with the tumor cells in Matrigel prior to injection. Daily i.p. injections of the test compound are made either on days 1-6 or days 7-13 after tumor inoculation.
20 The animals are killed about three or four weeks after tumor inoculation, and the lung tumor colonies are counted. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing and route of administration.

25 The activity of the compounds of the present invention toward decreasing tumor volume and metastasis can be evaluated in the model described by Rabbani et al. [Int. J. Cancer 63:840-845, 1995] to evaluate their inhibitor. There, Mat LyLu tumor cells over-expressing uPA were injected into
30 the flank of Copenhagen rats. The animals were implanted with osmotic minipumps to continuously administer various doses of test compound for up to three weeks. The tumor mass

and volume of experimental and control animals were evaluated during the experiment, as were metastatic growths.

Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing, and route of administration. Some of these authors described a related protocol in Xing et al. [Canc. Res. 57:3585-3593, 1997].

To evaluate the inhibitory activity of a compound of the present invention toward neovascularization, a rabbit cornea neovascularization model can be employed. Avery et al.

[Arch. Ophthalmol. 108:1474-1475, 1990] describe anesthetizing New Zealand albino rabbits and then making a central corneal incision and forming a radial corneal pocket. A slow release prostaglandin pellet was placed in the pocket to induce neovascularization. Test compound was administered i.p. for five days, at which time the animals were killed. The effect of the test compound is evaluated by review of periodic photographs taken of the limbus, which can be used to calculate the area of neovascular response and, therefore, limbal neovascularization. A decreased area of neovascularization as compared with appropriate controls indicates the test compound was effective at decreasing or inhibiting neovascularization.

An angiogenesis model used to evaluate the effect of a test compound in preventing angiogenesis is described by Min et al. [Canc. Res. 56:2428-2433, 1996]. C57BL6 mice receive subcutaneous injections of a Matrigel mixture containing bFGF, as the angiogenesis-inducing agent, with and without test compound. After five days, the animals are killed and the Matrigel plugs, in which neovascularization can be visualized, are photographed. An experimental animal receiving Matrigel and an effective dose of test compound will exhibit less vascularization than a control animal or an

experimental animal receiving a less- or non-effective dose of compound.

An *in vivo* system designed to test compounds for their ability to limit the spread of primary tumors is described by Crowley et al. [Proc. Natl. Acad. Sci. 90:5021-5025, 1993].
5 Nude mice are injected with tumor cells (PC3) engineered to express CAT (chloramphenicol acetyltransferase). The cells secrete large amounts of uPA and exhibit saturating amounts of uPA activity bound to uPAR on the cell surface. Compounds
10 to be tested for their ability to decrease tumor size and/or metastases are administered to the animals, and subsequent measurements of tumor size and/or metastatic growths are made. In addition, the level of CAT detected in various organs provides an indication of the ability of the test
15 compound to inhibit metastasis; detection of less CAT in tissues of a treated animal versus a control animal indicates less CAT-expressing cells migrated to that tissue.

In vivo experimental models designed to evaluate the urokinase inhibitory potential of a test compound, using a
20 tumor cell line F3II, said to be highly invasive, are described by Alonso et al. [Breast Canc. Res. Treat. 40:209-223, 1996]. This group describes *in vivo* studies for toxicity determination, tumor growth, invasiveness, spontaneous metastasis, experimental lung metastasis, and an
25 angiogenesis assay.

The CAM model (chick embryo chorioallantoic membrane model), first described by L. Ossowski in 1998 [J. Cell Biol. 107:2437-2445, 1998], provides another method for evaluating the urokinase inhibitory activity of a test compound. In the
30 CAM model, invasion of tumor cells through the chorioallantoic membrane is dependent upon the presence of catalytically active uPA. Contacting CAM with tumor cells in

the presence of a urokinase inhibitory agent results in less or no invasion of the tumor cells through the membrane.

Thus, the CAM assay is performed with CAM and tumor cells in the presence and absence of various concentrations of test
5 compound. The invasiveness of tumor cells is measured under such conditions to provide an indication of the compound's urokinase inhibitory activity. A compound having urokinase inhibitory activity correlates with less tumor invasion.

The CAM model is also used in a standard assay of
10 angiogenesis (i.e., effect on formation of new blood vessels (Brooks, P.C.; Montgomery, A.M.P.; and Cheresch, D.A., Methods in Molecular Biology 129: 257-269 (1999)). According to this model, a filter disc containing an angiogenesis inducer, such as basic fibroblast growth factor (bFGF) is placed onto the
15 CAM. Diffusion of the cytokine into the CAM induces local angiogenesis, which may be measured in several ways such as by counting the number of blood vessel branch points within the CAM directly below the filter disc. The ability of compounds of the present invention to inhibit cytokine-
20 induced angiogenesis can be tested using this model. A test compound can either be added to the filter disc that contains the angiogenesis inducer, be placed directly on the membrane or be administered systemically. The extent of new blood vessel formation in the presence and/or absence of test
25 compound can be compared using this model. The formation of fewer new blood vessels in the presence of a test compound would be indicative of anti-angiogenesis activity. Since certain of the compounds of the present invention are active as inhibitors of urokinase, anti-angiogenesis activity for
30 such compounds may suggest that urokinase plays a significant role in angiogenesis.

4. Pharmaceutical Compositions

In another aspect, the present invention encompasses pharmaceutical compositions prepared for storage or administration which comprise a therapeutically effective amount of a compound of the present invention in a pharmaceutically acceptable carrier.

The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

The therapeutically effective amount of the compound of the present invention can range broadly depending upon the desired affects and the therapeutic indication. Typically, dosages will be between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as

preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id.

The pharmaceutical compositions of the present invention may be formulated and used as tablets, capsules or elixirs
5 for oral administration; suppositories for rectal administration; sterile solutions and suspensions for injectable administration; and the like. The dose and method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent
10 medication and other factors which those skilled in the medical arts will recognize.

When administration is to be parenteral, such as intravenous on a daily basis, injectable pharmaceutical compositions can be prepared in conventional forms, either as
15 liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, or the like. In addition, if desired, the
20 injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (e.g., liposomes) may be utilized.

25 5. Utility

The compounds of the present invention having urokinase inhibitory activity and/or activity in reducing or inhibiting blood vessel formation, including angiogenesis and neovascularization, may be used both in vitro and in vivo for
30 a number of applications, some of which are described herein below.

The compounds of the present invention are active as inhibitors of urokinase and specifically bind urokinase. Accordingly those compounds that contain sites suitable for linking to a solid/gel support may be used in vitro for affinity chromatography to purify urokinase from a sample or to remove urokinase from a sample using conventional affinity chromatography procedures. These compounds are attached or coupled to an affinity chromatography either directly or through a suitable linker support using conventional methods. See, e.g. Current Protocols in Protein Science, John Wiley & Sons (J.E. Coligan et al., eds, 1997) and Protein Purification Protocols, Humana Press (S. Doonan, ed., 1966) and references therein.

The compounds of the present invention having urokinase inhibitory activity are useful in in vitro assays to measure tPA activity in a sample. In assays which measure the total plasminogen activation activity in a blood sample, a compound of the present invention having urokinase inhibiting activity will knock out that portion of plasminogen activation attributable to uPA, which will allow for calculation of the portion of the total plasminogen activation due to tPA activity as well as that due to uPA activity. Use of such assays to monitor tPA activity would allow better dosage control in patients receiving tPA. These assays could also be used to monitor uPA activity levels in tissue samples, such as from biopsy or to monitor uPA/tPA activities for any clinical situation where measurement of plasminogen activation activity is of assistance. These assays may also be used to monitor plasminogen activator activity where a patient has been treated with a non-endogenous compound having plasminogen activator activity, such as streptokinase and staphylokinase.

The compounds of the present invention are useful in vivo for treatment of pathologic conditions which would be ameliorated by decreased urokinase activity. For example these compounds will inhibit the activation of metalloproteases by the uPA-plasmin cascade in synovial fluid and thus, may be used in treatment of arthritis.

It is believed these compounds will be useful in decreasing or inhibiting metastasis, neovascularization, and degradation of the extracellular matrix in tumors and other neoplasms. These compounds will be useful as therapeutic agents in treating conditions characterized by pathological neovascularization such as retinal disease, retinopathies and other conditions, including those described hereinabove in the Background and Introduction to the Invention.

Another use for the compounds of the present invention having urokinase inhibitory activity is as an antidote if too much exogenous urokinase has been given to a patient for therewith purposes, such as for dissolving a blood clot.

The compounds of the present invention may be used in treating conditions characterized by inflammation due to their anti-inflammatory effects from inhibition of urokinase, thereby interfering with mediators of cell adhesion or migration. Such anti-inflammatory applications include treatment of stroke and complications of organ transplants.

The present invention includes methods for preventing or treating a condition in a mammal suspected of having a condition which will be attenuated by inhibition of urokinase activity comprising administering to said mammal a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

The compounds or pharmaceutical compositions of the present invention are administered in vivo, ordinarily in a

mammal, preferably in a human. In employing them *in vivo*, the compounds or pharmaceutical compositions can be administered to a mammal in a variety of ways, including orally, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms. Administration is preferably oral, such as by tablets capsules or elixirs taken on a daily basis.

In practicing the methods of the present invention, the compounds or pharmaceutical compositions of the present invention are administered alone or in combination with one another, or in combination with other therapeutic or *in vivo* diagnostic agents.

As is apparent to one skilled in the medical art, a "therapeutically effective amount" of the compounds or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, the particular mode of administration and the desired affects and the therapeutic indication. Because these factors and their relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the desired result of inhibiting uPA activity, will be within the ambit of one skilled in these arts. Typically, administration of the compounds or pharmaceutical composition of the present invention is commenced at lower dosage levels, with dosage levels being increased until the desired effect of inhibiting uPA activity to the desired extent is achieved, which would define a therapeutically effective amount. For the compounds of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.01

mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

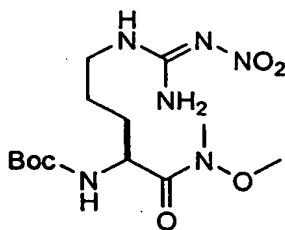
To assist in understanding, the present invention will now be further illustrated by the following examples. These examples as they relate to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the cope of the invention as described herein and hereinafter claimed.

Examples

A. Preparation Of Starting Material (See Figure 1)

15 Example 1

Preparation of N- α -t-butoxycarbonyl-N⁹-nitroargininyl-N-methoxy-N-methylamide



4-methylmorpholine (41.2 g, 407 mmol, 1.3 equiv.) was added to a solution of N- α -t-butoxycarbonyl-N⁹-nitroarginine (100 g, 313 mmol), N,O-dimethylhydroxylamine hydrochloride (61.2 g, 626 mmol, 2 equiv.), EDC (77.9 g, 407 mmol, 1.3 equiv.), and 1-hydroxybenzotriazole (55.1 g, 407 mmol, 1.3 equiv.) in anhydrous acetonitrile (523mL). The reaction mixture was stirred at room temperature over 16 hours and solvents were removed in vacuo. The residue was partitioned

between ethyl acetate and water. The aqueous phase was re-extracted with ethyl acetate (2 X). The combined organic layers were washed with 1N HCl (1 X), water (1 X), saturated sodium bicarbonate (1 X), water (1 X), and then dried

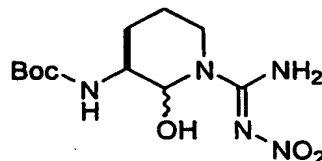
5 (magnesium sulfate), filtered, and concentrated in vacuo.

The title compound was obtained in 78% mass recovery as a white foam and was used without further purification. TLC of the products indicated satisfactory purity. $R_f = 0.21$ (10% methanol/ dichloromethane). MS ($M + H^+$) = 363.0, calculated
10 (MW) = 362.2.

1H NMR (400 MHz, $CDCl_3$): d 1.47 (s, 9H), 1.55-1.68 (m, 2H), 1.71-1.83 (br, 2H), 3.23 (s, 3H), 3.25-3.35 (m, 1H), 3.55-3.68 (m, 1H), 3.75-3.80 (s, 3H), 4.66 (t, 1H), 5.63 (d, 1H).

Example 2

15 Preparation of N- α -t-butoxycarbonyl-N⁹-nitro-argininal



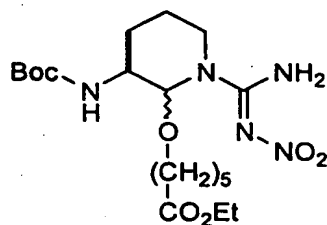
To a solution of the compound of Example 1 (25 g, 68.9 mmol, 1 equiv.) in anhydrous tetrahydrofuran (300 mL) in a 1 Liter 3-necked round bottomed flask, was added dropwise 1M
20 lithium aluminum hydride/tetrahydrofuran (100 mL, 1.45 equiv.) at -78 °C over 30 minutes under nitrogen atmosphere. The reaction mixture was kept at -78 °C with stirring for 1 hour, and then allowed to stir at room temperature for 20 to 30 minutes. A thick slurry was observed. The reaction
25 mixture was once again cooled to -78 °C and quenched slowly with 2M potassium bisulfate (100 mL). The precipitate was filtered out and washed with tetrahydrofuran (200 mL). The combined filtrate was concentrated in vacuo. The crude

residue was partitioned with ethyl acetate and water. The organic phases were washed with 0.5 N HCl, saturated sodium bicarbonate, and brine. The residue was dried over magnesium sulfate and filtered. Evaporation of the filtrate gave 15.9 g (76% yield) of the title compound as a white solid. $R_f = 0.13$ (50% hexane/ethyl acetate). The desired product was judged pure by TLC. MS: $(M + H^+) = 304.0$, calculated (MW) = 303.1. 1H NMR (400 MHz, $CDCl_3$): d 1.47 (s, 9H), 1.55-1.68 (m, 2H), 1.71-1.83 (m, 2H), 3.19-3.35 (m, 2H), 4.41-4.48 (m, 1H), 5.82 (s, 1H).

Alternatively, the title compound can be made following the procedures set forth in Example 2 of U.S. Patent No. 5,731,413.

Example 3

15 Preparation of N- α -t-butoxycarbonyl-N⁹-nitro-argininal (6-hexanoic acid ethyl ester) cyclol

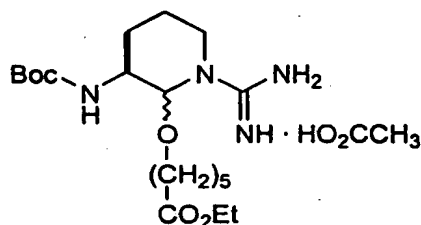


To a stirred solution of the compound of Example 2 (32.7 g, 107.9 mmol, 1 equiv.) and ethyl-6-hydroxyhexanoate (86.5 g, 539.6 mmol, 5 equiv.) in acetonitrile (20 mL), was added 3N HCl (260 μ L). All the reactants went into the solution after 15 minutes at room temperature. After the mixture was stirred over 24 hours, a small aliquot was taken for TLC and mass spectroscopy to determine completion. Then, acetic anhydride (55.1 g, 539.6 mmol, 5 equiv.) and pyridine (42.7 g, 539.6 mmol, 5 equiv.) were added into the reaction to cap the excess hydroxyester-linker. The reaction was

allowed to continue overnight. The residue was evaporated. The residue was taken up in ethyl acetate and washed with 1N HCl (1 X), water (1 X), saturated sodium bicarbonate (1 X), water (1 X), and dried (magnesium sulfate), filtered, and concentrated. The residue was purified by flash chromatography of silica gel, using 25-33% hexane/ethyl acetate gradient and afforded 45.0 g of the title compound (93.7% yield) as a viscous oil. $R_f = 0.24$ (50% hexanes/ethyl acetate). MS: $(M + H^+) = 446.0$, calculated (MW) = 445.2. 1H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.31-1.38 (m, 2H), 1.45 (s, 9H), 1.50-1.83 (m, 8H; aliph., b, g), 2.25-2.35 (m, 2H), 2.05-3.28 (m, 1.5H), 3.30-3.45 (m, 2H), 3.55-3.65 (br, .5H), 3.75-3.83 (br, 1H), 4.05-4.15 (m, 2H), 4.85 (d, 1H), 5.60 (s, 1H).

15 Example 4

Preparation of N- α -t-butoxycarbonyl-argininal (6-hexanoic acid ethyl ester) cyclol, acetate salt

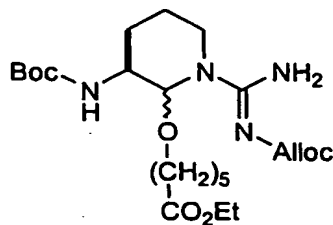


To a solution of the compound of Example 3 (45.8 g, 103.0 mmol, 1 equiv.) in ethanol/water/acetic acid (4:1:1) (200 mL) was added 10% palladium on carbon (9.2 g, 20% by weight). The mixture was hydrogenated at 40 psi for 16 hours. The solution was filtered, and the filtrate was evaporated in vacuo. The residue was taken in water and washed with ether (3 X). The organic layers were re-extracted with water (1 X). Aqueous phases were combined and lyophilized to afford the title compound (44.7 g, 94.4%

yield). $R_f = 0.18$ (dichloromethane/methanol/conc. ammonium hydroxide; 25:5:1). MS: $(M + H^+) = 401.0$, calculated (MW) = 400.2. 1H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.31-1.38 (m, 2H), 1.45 (s, 9H), 1.50-1.83 (m, 8H; aliph., b, g), 2.25-2.35 (m, 2H), 3.05-3.28 (m, 1.5H), 3.32-3.48 (m, 2H), 3.55-3.61 (br, .5H), 3.63-3.73 (m, 1H), 4.08-4.15 (m, 2H), 4.89 (d, 1H), 5.14 (d, 1H).

Example 5

Preparation of N- α -t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoic acid ethyl ester) cyclol

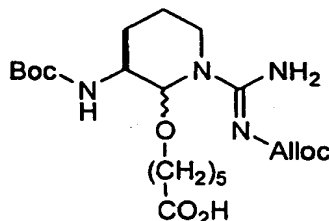


To a suspension of the compound of Example 4 (44.7 g, 97.3 mmol, 1 equiv.) in dichloromethane (292 mL) at 0 °C was added a solution of 1N sodium hydroxide (291.9 mL, 291.9 mmol, 3 equiv.) portion-wise to maintain pH = 11-13. Allylchloroformate (15.3 g, 126.5 mmol, 1.3 equiv.) was added in three-portions into the reaction. After monitoring the reaction by TLC and MS for 1 hour, the mixture was extracted with dichloromethane (3 X), dried (magnesium sulfate), filtered, and evaporated. The crude residue was purified immediately by flash chromatography of silica gel, using hexane and ethyl acetate as eluent. The title compound (41.5 g) was obtained (88.0% yield) as a viscous oil. $R_f = 0.30$ (50% hexanes/ethyl acetate) MS: $(M + H^+) = 485.0$, calculated (MW) = 484.2. 1H NMR (400 MHz, $CDCl_3$): δ 1.24 (t, 3H), 1.31-1.38 (m, 2H), 1.45 (s, 9H), 1.50-1.83 (m, 8H; aliph., b, g), 2.25-2.33 (m, 2H), 2.90-3.12 (m, 1.5H), 3.30-3.45 (m,

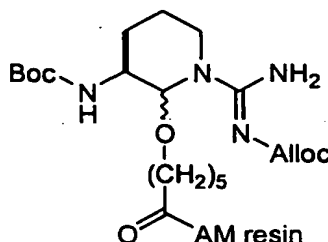
2H), 3.55-3.65 (br, .5H), 3.75-3.83 (br, 1H), 4.05-4.15 (m, 2H), 4.57 (d, 2H), 4.83 (s, 1H), 5.25 (q, 2H), 5.90-6.05 (m, 1H).

Example 6

5 Preparation of N- α -t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoic acid) cyclol



To a solution of the compound of Example 5 (40.3 g, 83.2 mmol) in ethanol (83.2 mL) was added 3N lithium hydroxide (55.5 mL, 166.4 mmol, 2 equiv.). Following stirring at room temperature for 2 1/2 hours, the reaction mixture was concentrated in vacuo. The residue was dissolved in water and washed with ether (3 X). The aqueous phase was acidified to pH = 2-3 with 1N HCl and extracted with dichloromethane. The solution was dried (magnesium sulfate), filtered, and evaporated to afford 31.5 g of the title compound (83.0% yield) as a white glassy foam. R_f = 0.33 (ethyl acetate). MS: $(M + H^+) = 457.1$, calculated (MW) = 456.2. 1H NMR (400 MHz, $CDCl_3$): d 1.45 (s, 9H), 1.52-1.87 (m, 10H; aliph., b, g), 2.32-2.43 (m, 2H), 3.05-3.28 (m, 1.5H), 3.37-3.55 (m, 2H), 3.83 (s, 1H), 4.57-4.65 (m, 2H), 4.88 (d, 1H), 5.31 (q, 2H), 5.53 (s, 1H), 5.90-6.05 (m, 1H).

Example 7Preparation of N- α -t-butoxycarbonyl-N- ω -allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

5 To aminomethylated polystyrene resin (22.6 g, 26.2 mmol, 1 equiv.), the compound of Example 6 (15.5 g, 34.0 mmol, 1.3 equiv.) and PyBOP (17.7 g, 34.0 mmol, 1.3 equiv.) in dimethylformamide (214 mL) at room temperature was added, followed by diisopropylethylamine (4.4 g, 34.0 mmol, 1.3 equiv.). The mixture was allowed to stir slowly in a round bottom flask overnight. The resin was washed with copious amounts of dichloromethane and methanol. The resin was dried under vacuum (a 3 mg sample of dried resin was taken out for Kaiser Test) and acetylated with dimethylformamide/acetic anhydride/triethylamine (8:1:1) for 30 minutes at ambient temperature. Once again, the resin was washed successively with organic solvents (dichloromethane and methanol), and dried under vacuum to afford the title compound (40.8 g; 92.0% yield by weight). Kaiser Test (O.D: 99% coupled); Resin substitution (approx. 0.75 mmol/g).

B. General Procedure For Solid-Phase Preparation Of Smaller Quantities Of Compounds

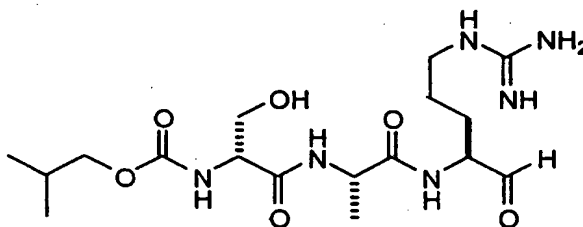
25 Solid-phase synthesis is useful for the production of small amounts of certain compounds of the present invention. As with the conventional solid-phase synthesis of peptides, reactors for the solid-phase synthesis of peptidyl argininals

are comprised of a reactor vessel with at least one surface permeable to solvent and dissolved reagents, but not permeable to synthesis resin of the selected mesh size. Such reactors include glass solid phase reaction vessels with a scintered glass frit, polypropylene tubes or columns with frits, or reactor KansTM made by Irori Inc., San Diego CA. The type of reactor chosen depends on volume of solid-phase resin needed, and different reactor types might be used at different stages of a synthesis.

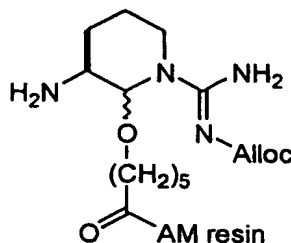
Example 8 describes a general procedure for solid-phase syntheses of small quantities of compounds of the present invention. See Figure 2. Modifications of this procedure to yield additional compounds of the present invention are described in Examples 9 to 15.

Example 8

Solid-Phase Synthesis of isobutyloxycarbonyl-D-seryl-L-
alanyl-argininal (Compound 1)

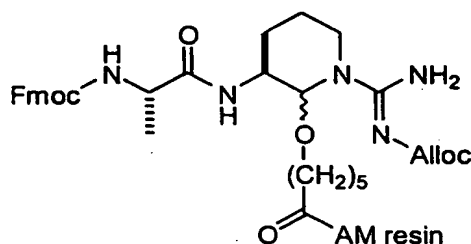


Step 1: Preparation of N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol



To a 60 mL solid phase reaction vessel was added 2.0 g of the compound of Example 7 (0.6-0.7 meq/g substitution), and a mixture of dichloromethane (12 mL), trifluoroacetic acid (6 mL), and thioanisole (2 mL). Nitrogen gas was bubbled for 15 minutes. The reactants were drained from the resin and the resin was washed successively with dichloromethane (2 X 20 mL), diisopropylethylamine (20 mL), dichloromethane (2 X 20 mL), diisopropylethylamine (20 mL), dichloromethane (2 X 20 mL), and diethyl ether (2 X 20 mL). The title compound was stored under vacuum. A ninhydrin test of the resin showed a dark blue color characteristic of the free amine produced by the removal of the t-t-butoxycarbonyl group.

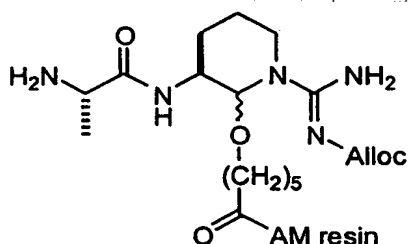
Step 2: Preparation of N- α -Fmoc-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol



The compound of Step 1 (2.1 g) was placed in a solid phase reaction vessel to which were added Fmoc-alanine (1 g, 3.2 mmol), 1-hydroxybenzotriazole (0.5 g, 3.2 mmol), TBTU (1.024 g, 3.2 mmol) and diisopropylethylamine (600 μ L, 3.4 mmol) in dimethylformamide (15-20 mL). Nitrogen gas was bubbled through the reactor at room temperature for 2 hours. The reagents were drained from the resin and the resin was washed successively with dimethylformamide (2 X 20 mL), dichloromethane (2 X 20 mL), dimethylformamide (2 X 20 mL), dichloromethane (2 X 20 mL), and diethyl ether (2 X 20 mL).

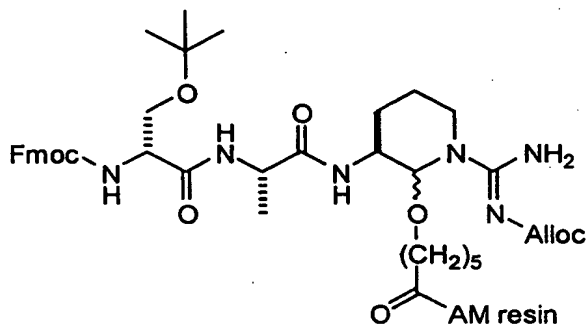
The resin was vacuum dried and a small aliquot was taken for ninhydrin colorimetric analysis, which showed a 99.5% coupling efficiency in the production the title compound.

Step 3: Preparation of alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol



The compound of Step 2 was treated with 50% piperidine in dimethylformamide (20 mL) for 30 minutes at room temperature with nitrogen gas agitation. The resin was washed as above and vacuum dried to give the title compound. A ninhydrin assay on a small aliquot gave dark blue resin and solution showing a high yield for the deprotection.

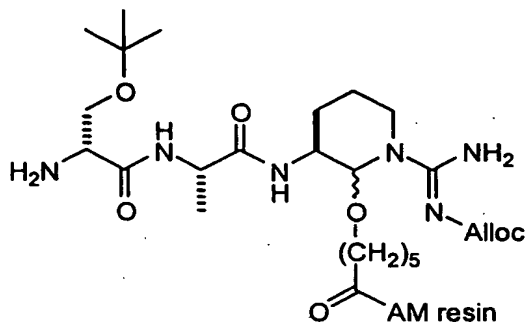
Step 4: Preparation of N-α-Fmoc-D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol



The compound of Step 3 (100 mg) was placed in an Irori KanTM reaction vessel. The vessel was placed in a 20 mL vial

containing dimethylformamide (4 mL), N- α -Fmoc-D-serine(O-t-butyl) (184 mg, 0.48 mmol), 1-hydroxybenzotriazole (73 mg, 0.48 mmol), TBTU (154 mg, 0.48 mmol), and diisopropylethylamine (84 μ l, 0.48 mmol). The vessel was
5 agitated for 3 hours on a shaker table. The vessel was drained, washed successively with dimethylformamide (2 X 3 mL), dichloromethane(2 X 3 mL), dimethylformamide(2 X 3 mL), dichloromethane(2 X 3 mL), isopropanol(2 X 3 mL), dichloromethane(2 X 3 mL), isopropanol(2 X 3 mL), and diethyl
10 ether(2 X 3 mL). The resin was dried under vacuum to give the title compound.

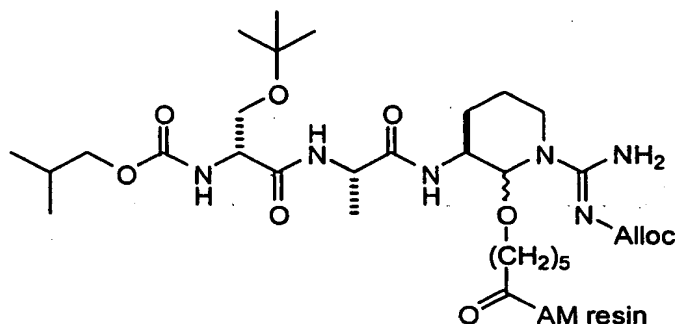
Step 5: Preparation of D-seryl(O-t-butyl)-alanyl-N-
omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated
polystyrene resin) cyclol



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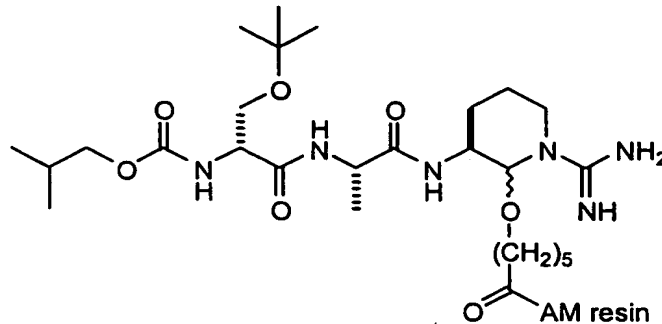
The vessel containing the compound of Step 4 was treated with 50% piperidine in dimethylformamide (5 mL) in a 20 mL vial for 45 minutes at room temperature while agitated on a shaker table. The resin was washed as above and vacuum dried
20 to give the compound of Step 5. A ninhydrin assay on a small aliquot gave a dark blue resin and solution indicating a high yield for the deprotection.

Step 6: Preparation of carbamate analogs of D-seryl (O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol such as:



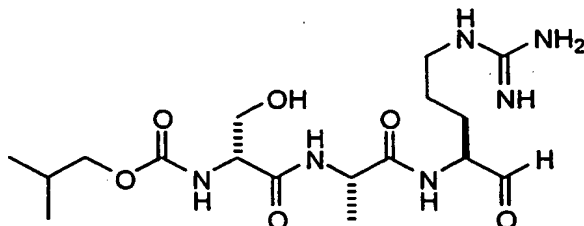
- 5 The vessel containing the compounds of Step 5, along with other vessels containing related resin-bound analogs (see Examples 9 to 14, below), were/are each placed in a 20 mL vial with 0.12M various individual chloroformates, including isobutylchloroformate, in dimethylformamide (5-10
- 10 mL). Diisopropylethylamine (105-210 μ L, 0.6-1.2 mmol) was/is added, and the vials were/are shaken for 2.5 hours. All vessels are drained and washed successively with dimethylformamide (2 X 3 mL), dichloromethane (2 X 3 mL), dimethylformamide (2 X 3 mL), dichloromethane (2 X 3 mL),
- 15 isopropanol (2 X 3 mL), dichloromethane (2 X 3 mL), isopropanol (2 X 3 mL), and diethyl ether (2 X 3 mL). The KansTM were/are vacuum dried, including the one containing isobutyloxycarbonyl-D-seryl (O-t-butyl)-alanyl-N- ω -allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated
- 20 polystyrene resin) cyclol.

Step 7: Preparation of carbamate analogs of D-seryl(O-t-butyl)-alanyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol such as:



- 5 Removal of the allyloxy protecting group from the products of Step 5 was/is accomplished for several compounds of the present invention simultaneously by placing a collection of 38 vessels in a 250 mL polypropylene bottle and adding a mixture of methylsulfoxide (10 mL), tetrahydrofuran
- 10 (10 mL), 1N HCl (2.5 mL), and morpholine (25 mL). Tetrakis triphenylphosphine palladium (0.87 g) was/is then added, and the bottle was/is shaken for 4 hours at room temperature. The vessels, including the one containing
- 15 isobutyloxycarbonyl-D-seryl(O-t-butyl)-alanyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol, were/are drained, washed successively with dimethylformamide (2 X 3 mL), dichloromethane(2 X 3 mL), dimethylformamide(2 X 3 mL), dichloromethane(2 X 3mL), isopropanol(2 X 3 mL),
- 20 dichloromethane(2 X 3 mL), isopropanol(2 X 3 mL), and diethyl ether(2 X 3 mL), and vacuum dried to give individual title compounds in vessels.

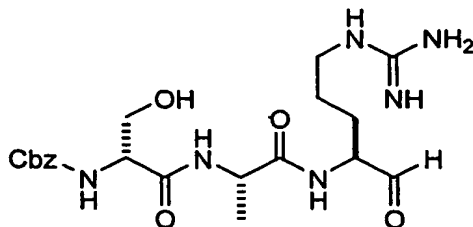
Step 8: Preparation of isobutyloxycarbonyl-D-seryl-
alanyl-argininal



The vessel containing isobutyloxycarbonyl-D-seryl (O-t-
5 butyl)-alanyl-argininal (6-hexanoyl-aminomethylated
polystyrene resin) cyclol was emptied into a Whatman
polypropylene mini-column containing trifluoroacetic
acid/dichloromethane/water (1.5 mL of a 6:3:1 mixture). The
column was shaken for 4 hours at room temperature. The
10 reaction solution was drained into a test tube and the resin
was washed with dichloromethane and water. The wash mixtures
and the reaction mixtures were collected in the same test
tube, and the mixture was agitated, then allowed to separate
into two phases. The water layer was removed and filtered.
15 The title compound (Compound 1) was purified by
semipreparative reverse-phase HPLC, lyophilized, and weighed,
and analyzed by HPLC and mass spectrometry. An alternate
route to synthesis of Compound 1 is provided in Examples 36
through 43.

20 Example 9

Solid-phase synthesis of benzyloxycarbonyl-D-seryl-alanyl-
argininal (Compound 2)



Steps 1 through 4 and 7 through 8 of Example 8 are followed to make benzyloxycarbonyl-D-seryl-alanyl-argininal, compound 2, with the substitution in Step 4 of Cbz-D-serine(O-t-butyl) for Fmoc-D-serine(O-t-butyl).

5 Example 10

Solid-phase synthesis of Compounds 3 to 9 and 35 to 40

Steps 1 through 8 of Example 8 are followed to make the following compounds, with the noted substitution for isobutylchloroformate in Step 6:

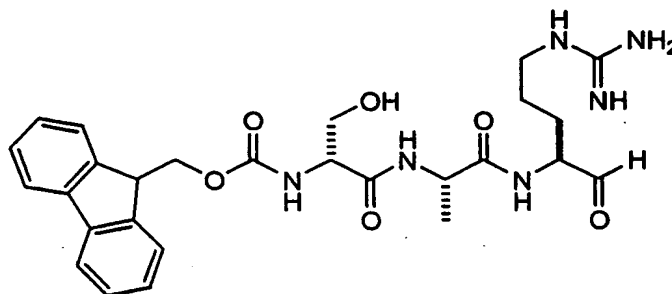
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COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 6
3	(-)-menthyloxy-carbonyl-D-seryl-alanyl-argininal	(-)-menthylchloroformate
4	2-naphthyloxy-carbonyl-D-seryl-alanyl-argininal	2-naphthylchloroformate
5	phenoxycarbonyl-D-seryl-alanyl-argininal	phenylchloroformate
6	2-ethylhexyloxy-carbonyl-D-seryl-alanyl-argininal	2-ethylhexylchloroformate
7	(+)-menthyloxy-carbonyl-D-seryl-alanyl-argininal	(+)-menthylchloroformate
8	methyloxycarbonyl-D-seryl-alanyl-argininal	methylchloroformate
9	n-butyloxycarbonyl-D-seryl-alanyl-argininal	n-butylchloroformate
35	3-cyclohexylpropionyl-D-seryl-alanyl-argininal	3-cyclohexylpropionyl chloride

36	cyclohexylmethyloxycarbonyl-D-seryl-alanyl-argininal	cyclohexylmethyl chloroformate
37	cyclohexylmethylazacarbonyl-D-seryl-alanyl-argininal	cyclohexylmethyl isocyanate
38	benzylazacarbonyl-D-seryl-alanyl-argininal	benzyl isocyanate
39	isovaleryl-D-seryl-alanyl-argininal	isovaleryl chloride
40	isopropyloxycarbonyl-D-seryl-alanyl-argininal	isopropyl chloroformate

Example 11

Solid-phase synthesis of fluorenylmethyloxycarbonyl-D-seryl-alanyl-argininal (Compound 10)



5 Steps 1 through 4 and Steps 7 and 8 of Example 8 are followed to make fluorenylmethyloxycarbonyl-D-seryl-alanyl-argininal, Compound 10.

Example 12

Solid-phase synthesis of compounds 11 to 18

10 Steps 1 through 4 and 7 through 8 of Example 8 are followed to make Compounds 11 to 18, with the substitution in Step 4 of Cbz-D-serine(O-t-butyl) for Fmoc-D-serine(O-t-

butyl), and the noted substitution in Step 2 for Fmoc-alanine.

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 2
11	benzyloxycarbonyl-D-seryl-prolyl-argininal	Fmoc-proline
12	benzyloxycarbonyl-D-seryl-L-azetidine-2-carbonyl-argininal	Fmoc-L-azetidine-2-carbocyclic acid
13	benzyloxycarbonyl-D-seryl-pipecolyl-argininal	Fmoc-pipecolic acid
14	benzyloxycarbonyl-D-seryl-2-aminobutanoyl-argininal	Fmoc-2-aminobutanoic acid
15	benzyloxycarbonyl-D-seryl-S-methylcysteinyl-argininal	Fmoc-S-methylcysteine
16	benzyloxycarbonyl-D-seryl-2-norvalyl-argininal	Fmoc-norvaline
17	benzyloxycarbonyl-D-seryl-2-glycyl-argininal	Fmoc-glycine
18	benzyloxycarbonyl-D-seryl-sarcosyl-argininal	Fmoc-sarcosine

Example 13

Solid-phase synthesis of Compounds 19 to 23

- 5 Steps 1 through 4 and Steps 7 through 8 of Example 8 are followed to make Compounds 19 to 23, with the noted substitution in Step 4 for Fmoc-D-serine(O-t-butyl).

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 4
19	benzyloxycarbonyl-D- allothreonyl-alanyl- argininal	Cbz-D- allothreonine (O- t-butyl)
20	benzyloxycarbonyl- threonyl-alanyl-argininal	Cbz-threonine (O- t-butyl)
21	benzyloxycarbonyl-D- alanyl-alanyl-argininal	Cbz-D-alanine
22	benzyloxycarbonyl-D- threonyl-alanyl-argininal	Cbz-D- threonine (O-t- butyl)
23	benzyloxycarbonyl-seryl- alanyl-argininal	Cbz-serine (O-t- butyl)

Example 14Solid-phase synthesis of Compounds 24, 25, and 33

Steps 1 through 4 and 7 through 8 of Example 8 are followed to make Compounds 24, 25, and 33 with the substitution in Step 4 of Cbz-D-allothreonine (O-t-butyl) for Fmoc-D-serine (O-t-butyl), and the noted substitution in Step 2 for Fmoc-alanine.

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 2
24	benzyloxycarbonyl-D- allothreonyl-sarcosyl- argininal	Fmoc-sarcosine
25	benzyloxycarbonyl-D- allothreonyl-N- methylalanyl-argininal	Fmoc-N- methylalanine

Example 15Solid-phase synthesis of Compounds 26 to 27

Steps 1 through 4 and 7 through 8 of Example 8 are followed to make Compounds 26 to 27, with the substitution in Step 2 of Fmoc-proline for Fmoc-alanine, and the noted substitution in Step 3 for Fmoc-D-serine(O-t-butyl).

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 4
26	benzyloxycarbonyl-D- threonyl-prolyl- argininal	Cbz-D-threonine(O- t-butyl)
27	benzyloxycarbonyl-D- homoseryl-prolyl- argininal	Cbz-D- homoserine(O-t- butyl)

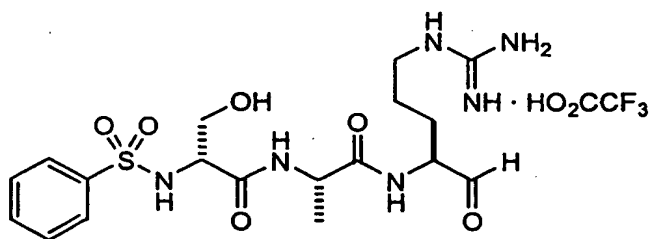
Example 16Solid-phase synthesis of Compounds 41 to 43

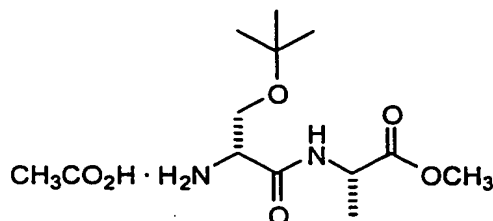
Steps 1 through 4 and 7 through 8 of Example 8 are followed to make Compounds 41 to 43, with the substitution in Step 4 of N- α -Cbz-N- β -Fmoc-D-2,3-diaminopropionic acid for Fmoc-D-serine(O-t-butyl), and the noted substitution in Step 6 for the individual chloroformates:

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 6
41	N- α -benzyloxycarbonyl-N- β -2-phenylethylcarbonyl-D-2,3-diaminopropionyl-alanyl-argininal	hydrocinnamoyl chloride
42	N- α -N- β -dibenzyloxycarbonyl-D-2,3-diaminopropionyl-alanyl-argininal	benzyl chloroformate
43	N- α -benzyloxycarbonyl-N- β -methyloxycarbonyl-D-2,3-diaminopropionyl-alanyl-argininal	methyl chloroformate

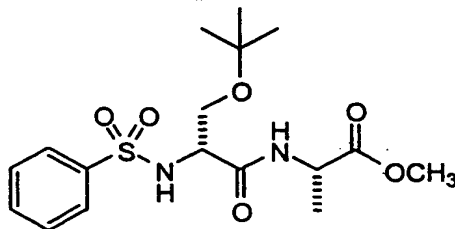
C. Alternate Synthetic Routes For Certain Compounds

(i) Examples 17 to 21 describe the synthesis of benzenesulfonyl-D-Ser-L-Ala-L-Arg-al, trifluoroacetate salt (Compound 28). See Figure 3.

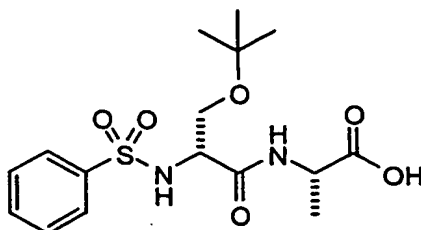


Example 17Synthesis of D-Ser(O-t-Bu)-Ala-OMe, acetate salt

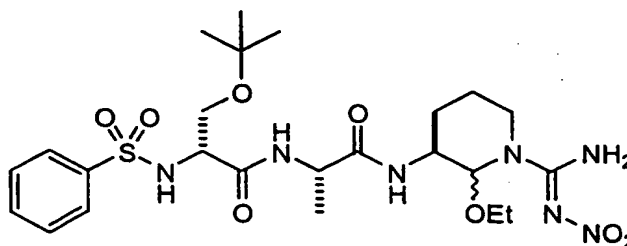
N- α -Cbz-D-serine (Bachem, 4.97 g, 16.8 mmol), alanine
5 methyl ester, hydrochloride salt (Novabiochem, 4.7 g, 33.7
mmol), EDC (6.5g, 33.7 mmol), and 1-hydroxybenzotriazole
(2.6g, 16.8 mmol) were combined, and acetonitrile (67 mL) was
added. After stirring as a slurry for 10 minutes,
diisopropylethylamine (14.4 mL, 84 mmol) was added, and the
10 resulting clear mixture was stirred for an additional 18
hours. The solvent was removed under reduced pressure, and
the residue was suspended in ethyl acetate (500 mL). The
solution was washed with 0.5M HCl (2 x 100 mL), followed by
saturated sodium bicarbonate (2 x 100 mL), and brine (100
15 mL). The organic layer was then dried with sodium sulfate,
and the solvent was removed in vacuo to afford Cbz-D-Ser(O-t-
Bu)-Ala-OMe in a quantitative yield as a single peak by
reverse-phase (C18) HPLC t_R =16.9 at 0.1% trifluoroacetic acid
in 5-75% aqueous acetonitrile over 20 minutes. Cbz-D-Ser(O-
20 t-Bu)-Ala-OMe was then dissolved in ethanol/acetic acid/water
(150 mL of a 4:1:1 mixture). The flask was charged with
nitrogen, and 10% palladium on carbon (1.5 g) was added.
This mixture was hydrogenated at 45 psi for 2 hours. The
palladium catalyst was filtered, and solvent removed under
25 reduced pressure to give 4.58 g of the title compound in a
95% yield as a single peak by reverse-phase (C18) HPLC
(t_R =8.0 minutes at 0.1% trifluoroacetic acid in 5-75% aqueous
acetonitrile over 20 minutes), and MS ($M+H$ =247.2).

Example 18Synthesis of benzenesulfonyl-D-Ser(O-t-Bu)-Ala-OMe

To a stirred slurry of the compound of Example 17 (1.0
5 g, 3.3 mmol) in acetonitrile (13 mL) was added benzenesulfonyl
chloride (0.87 g, 4.9 mmol). To this mixture
diisopropylethylamine (1.67 mL, 9.8 mmol) was added in five
portions over a 1 hour period. The mixture was allowed to
stir an additional hour. The solvent was removed under
10 reduced pressure, and the residue was suspended in ethyl
acetate (100 mL). The solution was washed with 0.5M HCl (2 x
10 mL), followed by saturated sodium bicarbonate (2 x 10 mL),
and brine (1 x 10 mL). The organic layer was then dried with
sodium sulfate, and solvent was removed under reduced
15 pressure. The residue was purified by flash chromatography
eluting with 50% hexanes/ethyl acetate, yielding 0.54 g, 1.4
mmol, of product in a 43% yield. The product was a single
peak by reverse phase (C18) HPLC (t_R =20.2 minutes at 0.1%
trifluoroacetic acid in 5-75% aqueous acetonitrile over 20
20 minutes). ^1H NMR (CD_3OD): 7.5-7.9 ppm (m, 5H), 4.3 ppm (q, 1H),
3.9 ppm (t, 1H), 3.7 (s, 3H), 3.4 ppm (m, 1H), 3.5 ppm (m, 1H),
1.3 ppm (d, 3H), 1.05 ppm (s, 9H).

Example 19Synthesis of benzenesulfonyl-D-Ser(O-t-Bu)-Ala-OH

To the compound of Example 18 (0.53 g, 1.4 mmol) in
 5 methanol (9 mL) was added 1.0M lithium hydroxide (3.0 mL, 3
 mmol). After stirring for 18 hours, the reaction mixture was
 poured over a column of 10 mL of DOWEX (50 X 8-400) ion
 exchange resin, and eluted with methanol/water (60 mL of a
 1:1 mixture). The methanol was pumped off under reduced
 10 pressure and the remaining water was lyophilized, yielding
 0.49 g, 1.3 mmol (95%) of the title compound as a single peak
 by reverse-phase (C18) HPLC (t_R =13.5 minutes at 0.1%
 trifluoroacetic acid in 5-75% aqueous acetonitrile over 20
 minutes). ^1H NMR(CD_3OD): 7.9ppm(d,2H), 7.6ppm(t,1H),
 15 7.5ppm(t,2H), 4.25ppm(q,1H), 3.9(t,1H), 3.5ppm(m,1H),
 3.4ppm(m,1H), 1.3ppm(d,3H), 1.075ppm(s,9H).

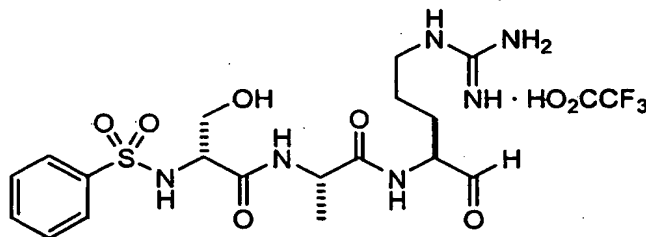
Example 20Synthesis of benzenesulfonyl-D-Ser(O-t-Bu)-Ala-N⁹-
nitroargininal ethyl cyclol

20 The compound of Example 19 (0.48 g, 1.3 mmol), L-N⁹-
 nitroargininal ethyl cyclol, hydrochloride salt (0.41g, 1.5
 mmol), EDC (0.37 g, 1.9 mmol), and 1-hydroxybenzotriazole

(0.20 g, 1.3 mmol) were combined and acetonitrile (5 mL) was added. After stirring the resulting slurry for 10 minutes, diisopropylethylamine (1.10 mL, 84 mmol) was added and the resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 mL). The solution was washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL), and brine (1 x 10 mL). The organic layer was dried with sodium sulfate, and solvent was removed in vacuo to afford 0.72 g the title compound in 95% yield. The product was a single peak by reverse phase (C18) HPLC (t_R =15.2 minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes), with MS($M+H$ =371).

15 Example 21

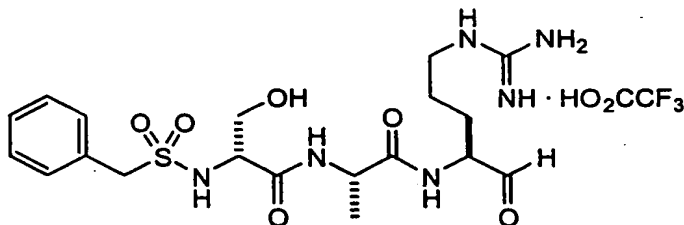
Synthesis of benzenesulfonyl-D-Ser-L-Ala-L-Arg-al,
trifluoroacetate salt (Compound 28)



The compound of Example 20 (0.72 g, 1.23 mmol) was hydrogenated over 10% palladium on carbon (250 mg) in ethanol/acetic acid/water (24.5 mL of 4:1:1 mixture) at 50 psi for 18 hours. The catalyst was filtered, and the solvent was removed from the filtrate in vacuo to give a quantitative yield of benzylsulfonyl-D-Ser(O-t-Bu)-L-Ala-L-argininal ethyl cyclol as a single peak by reverse-phase (C18) HPLC (t_R =12.8 minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes), and MS($M+H$ =541).

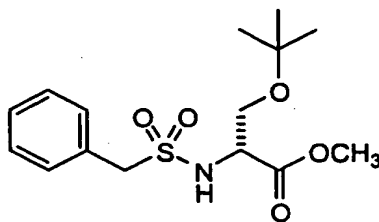
Benzylsulfonyl-D-Ser(O-t-Bu)-L-Ala-L-argininal ethyl cyclol was treated with 6M HCl (12.25 mL), stirred for 1 hour, then neutralized with 6.5M ammonium acetate to pH 4. This material was loaded directly onto a preparative HPLC column and eluted with a 0-20% aqueous acetonitrile gradient to afford 88 mg of the title compound in a 15% yield after lyophilization. The title compound had three peaks by reverse-phase (C18) HPLC (t_R =8.2 minutes, 9.2 minutes, and 9.5 minutes at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes), with MS ($M+H$ =457).

(ii) Examples 22 to 26 describe the synthesis of benzylsulfonyl-D-Ser-L-Ala-L-Arg-al, trifluoroacetate salt (Compound 29).



15 Example 22

Synthesis of benzylsulfonyl-D-Ser(O-t-Bu)-OMe

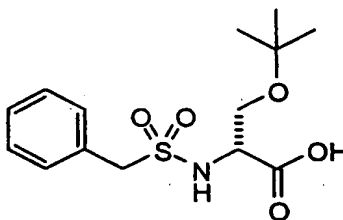


To a stirred solution of D-serine(O-t-Bu) methyl ester, hydrochloride salt (2.07 g, 9.8 mmol) in acetonitrile (39 mL) was added α -toluenesulfonyl chloride (1.86 g, 9.8 mmol). To this mixture of diisopropylethylamine (3.7 mL, 21.5 mmol) were added in five portions over a 1 hour period. The mixture was allowed to stir an additional hour. The solvent

was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 mL). The solution was washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL), and brine (1 x 10 mL). The organic
5 layer was dried with sodium sulfate, and solvent was removed in vacuo to give 2.84 g of the title compound in 88% yield. $R_f=0.4$ (4:1 ethyl acetate:hexanes).

Example 23

Synthesis of benzylsulfonyl-D-Ser(O-t-Bu)-OH

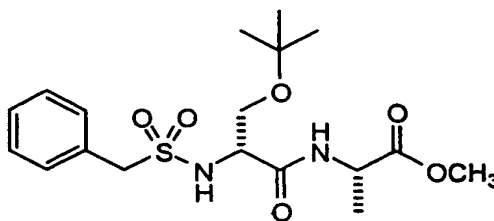


10

To a stirred solution of the compound of Example 22 (2.66 g, 8.1 mmol) in methanol (54 mL) was added 1.0M lithium hydroxide (17.8 mL, 17.8 mmol). The reaction mixture was allowed to stir for 18 hours, then poured over a column of 10
15 mL of DOWEX(50 X 8-400) ion exchange resin and eluted with methanol:water (60 mL of a 1:1 mixture). The methanol was removed under reduced pressure, and the remaining aqueous solution was lyophilized to afford 2.47 g of the title compound in 97% yield. $t_R=14.8$ minutes (0.1% trifluoroacetic
20 acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 24

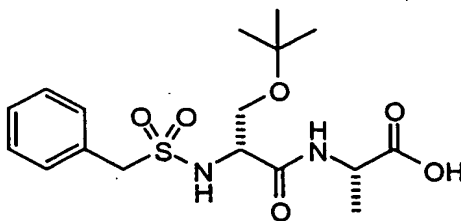
Synthesis of benzylsulfonyl-D-Ser(O-t-Bu)-Ala-OMe



The compound of Example 23 (1.0 g, 3.2 mmol), alanine methyl ester, hydrochloride salt (Novabiochem, 0.89 g, 6.3 mmol), EDC (1.22 g, 6.3 mmol), and 1-hydroxybenzotriazole (0.49 g, 3.2 mmol) were combined and acetonitrile (13 mL) was added. After stirring the resulting slurry for 10 minutes, diisopropylethylamine (2.71 mL, 15.8 mmol) was added and the resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 mL). The solution was washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL), and brine (1 x 10 mL). The organic layer was then dried with sodium sulfate, and solvent was removed in vacuo to afford 1.22 g of the title compound in 97% yield. t_R =16.2 minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 25

Synthesis of benzylsulfonyl-D-Ser(O-t-Bu)-Ala-OH



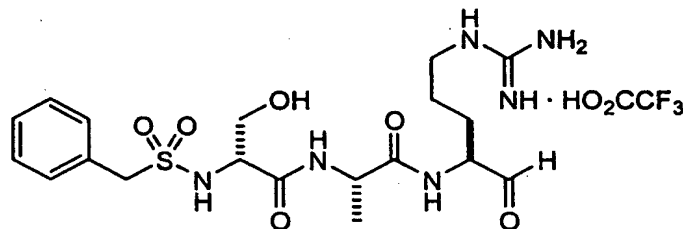
To the compound of Example 24 (1.22 g, 3.1 mmol) in methanol (22 mL), was added 1M lithium hydroxide (7.2 mL, 7.2 mmol). After stirring 18 hours, the reaction mixture was poured over a column of 10 mL of DOWEX(50 X 8-400) ion exchange resin and eluted with methanol:water (60 mL of a 1:1 mixture). The methanol was removed under reduced pressure, and the aqueous solution was lyophilized to afford 1.16 g of the title compound in 91% yield. t_R =13.2 minutes (0.1%

trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 26

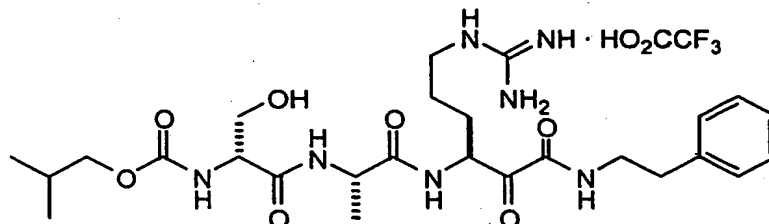
Synthesis of benzylsulfonyl-D-Ser-Ala-Arg-al,

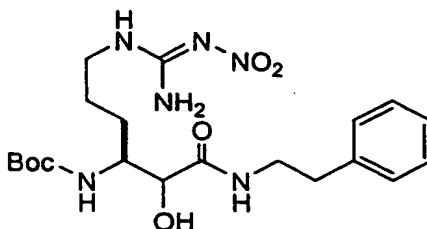
5 trifluoroacetate salt (Compound 29)



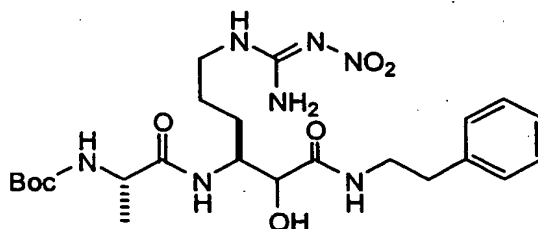
The title compound was synthesized from the compound of Example 25 following the procedures of Examples 20 and 21, with the compound of Example 25 replacing the compound of Example 19 in the procedure described in Example 20.

(iii) Examples 27 to 34 describe the synthesis of i-butoxycarbonyl-D-Ser-Ala-Arg-CO-phenethylamide, trifluoroacetate salt (Compound 30). See Figure 4.

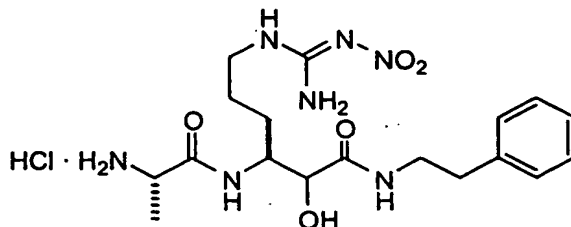


Example 27Synthesis of N- α -t-butoxycarbonyl-N⁹-nitroArg-COH-phenethylamide

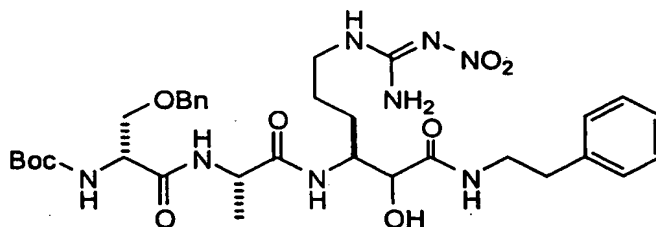
- 5 N- α -t-butoxycarbonyl-N⁹-nitroArg-COH-COOH (1.07 g, 3 mmol) (prepared following the procedure in US 5,371,072, Examples 2 to 5), phenethylamine (38 mL, 3 mmol), BOP (1.35 g, 3 mmol), and 1-hydroxybenzotriazole (0.23 g, 1.5 mmol) were combined and dimethylformamide (122 mL) was added. This
- 10 mixture was stirred for 10 minutes, followed by addition of 4-methylmorpholine (2.01 mL, 18 mmol), and the resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced pressure, and the residue was suspended in 100 mL of ethyl acetate. The solution was
- 15 washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL), and brine (1 x 10 mL). The organic layer was then dried with sodium sulfate, and solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with ethyl acetate:hexanes
- 20 (4:1) to afford 1.22 g of the title compound in 89% yield. t_R =13.2 minutes and 14.1 minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 28Synthesis of t-butoxycarbonyl-Ala-N^g-nitroArg-COH-phenethylamide

5 To a stirred solution of the compound of Example 27 (1.22 g, 2.7 mmol) in ethyl acetate (10 mL), was added 5M HCl in ethyl acetate (10 mL). After 2 hours the solid that formed was filtered, dried to give quantitative yields of N^g-nitroArg-COH-phenethylamide, hydrochloride salt, which was
10 combined with t-butoxycarbonyl-Ala-OH (Novabiochem, 0.77 g, 4.1 mmol), EDC (2.4 g, 5.4 mmol), and 1-hydroxybenzotriazole (0.42 g, 2.7 mmol); then, acetonitrile (11 mL) was added. This mixture was stirred as a slurry for 10 minutes and diisopropylethylamine (2.3 mL, 13.55 mmol) was added. The
15 resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced pressure and the residue was suspended in ethyl acetate (100 mL). The solution was washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL) and brine (1 x 10
20 mL). The organic layer was then dried with sodium sulfate, and solvent was removed in vacuo to afford 1.34 g of the title compound in a 95% yield. t_R =13.0 minutes and 13.6 minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 29Synthesis of HCl-Ala-N^g-nitroArg-COH-phenethylamide

The compound of Example 28 (1.34 g, 2.6 mmol) was dissolved in 10 mL of ethyl acetate. To this mixture 10 mL of 5M HCl in ethyl acetate were added and the resulting mixture stirred. After 2 hours the solid that formed was filtered and dried to give 1.188 g of the title compound in 98% yield.

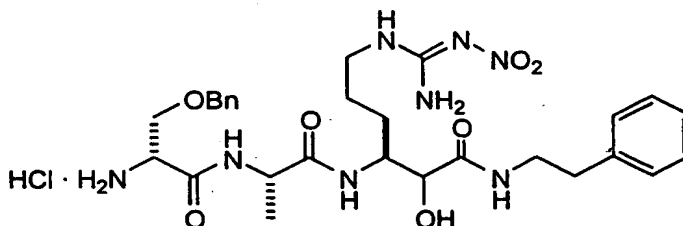
10 Example 30Synthesis of t-butoxycarbonyl-D-Ser(OBn)-Ala-N^g-nitroArg-COH-phenethylamide

The compound of Example 29 (1.18 g, 2.4 mmol), t-butoxycarbonyl-D-serine benzylester (Novabiochem, 1.08 g, 3.6 mmol), EDC (2.15 g, 4.9 mmol), and 1-hydroxybenzotriazole (0.37 g, 2.4 mmol) were combined, and acetonitrile (10 mL) was added. To this slurry was added diisopropylethylamine (2.1 mL, 15 mmol) over a 10 minute period, and the resulting clear mixture was stirred for an additional 18 hours. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 mL). The solution was washed with 0.5M HCl (2 x 10 mL), followed by saturated

sodium bicarbonate (2 x 10 mL), and brine (1 x 10 mL). The organic layer was then dried with sodium sulfate, and solvent was removed in vacuo to afford 1.56 g of the title compound in 92% yield. t_R =16.0 minutes and 16.5 minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes).

Example 31

Synthesis of HCl-D-Ser(OBn)-Ala-N^g-nitroArg-COH-phenethylamide



10

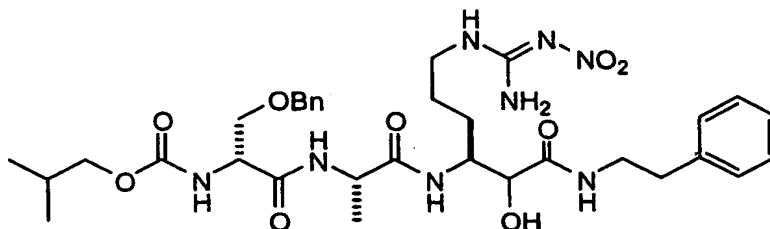
To a solution of the compound of Example 30 (1.5 g, 2.1 mmol) in ethyl acetate (10 mL) was added 5M HCl in ethyl acetate (10 mL). After stirring 2 hours, the solid that formed was filtered and dried to give 1.31 g of the title compound in 98% yield. t_R =11.8 minutes and 12.3 minutes at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

15

Example 32

Synthesis of i-butoxycarbonyl-D-Ser(OBn)-Ala-N^g-nitroArg-COH-phenethylamide

20

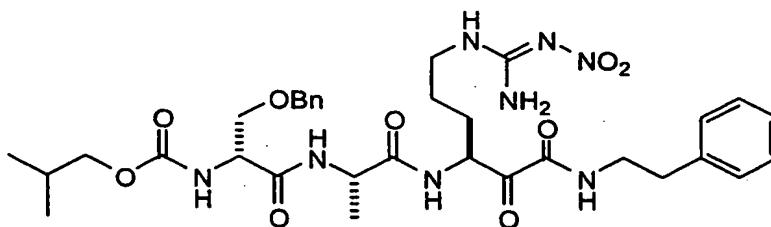


To a stirred solution of the compound of Example 31 (1.0 g, 1.6 mmol) in acetonitrile (6 mL), was added

isobutylchloroformate (0.225 mL, 1.8 mmol), followed by diisopropylethylamine (0.81 mL, 4.74 mmol); this mixture was stirred for 2 hours. The solvent was removed under reduced pressure, and the residue was suspended in ethyl acetate (100 mL). The solution was washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL), and brine (1 x 10 mL). The organic layer was then dried with sodium sulfate and solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with ethyl acetate/hexanes (4:1) to afford 0.94 g of the title compound in 85% yield. t_R =20.2 minutes and 21.2 minutes (0.1% trifluoroacetic acid in 25-45% aqueous acetonitrile over 20 minutes).

Example 33

Synthesis of i-butoxycarbonyl-D-Ser(OBn)-Ala-N⁹-nitroArg-CO-phenethylamide



The compound of Example 32 (0.1 g, 0.143 mmol) was dissolved in DMSO (1.4 mL). EDC (0.14 g, 0.714 mmol) and dichloroacetic acid (0.12 mL, 1.43 mmol) were added and the mixture stirred for 1 hour. The reaction mixture was then poured into ethyl acetate (50 mL) and washed with 0.5M HCl (2 x 10 mL), followed by saturated sodium bicarbonate (2 x 10 mL) and brine (1 x 10 mL). The organic layer was then dried with sodium sulfate and solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with ethyl acetate/hexanes (4:1) to afford 68 mg of

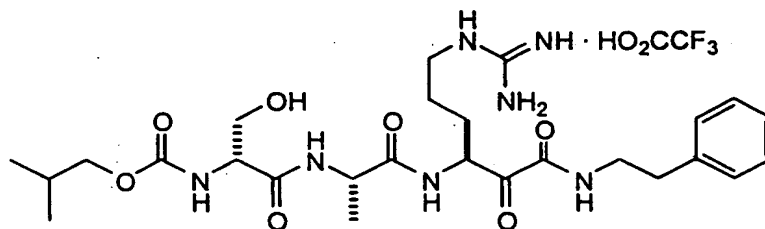
95

the title compound in 68% yield. $R_f=0.32$ (4:1 ethyl acetate/hexanes)

Example 34

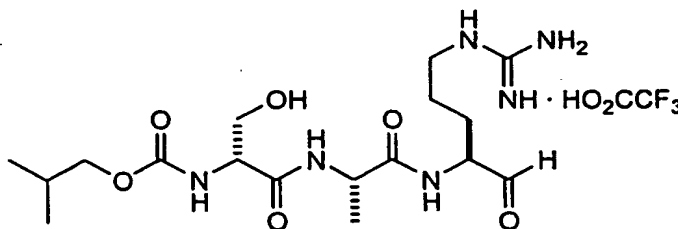
Synthesis of i-butoxycarbonyl-D-Ser-Ala-Arg-CO-

5 phenethylamide, trifluoroacetate salt

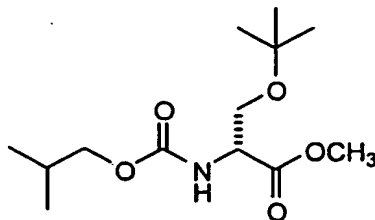


The compound of Example 33 (68 mg, 0.1 mmol) and anisole (1 mL) were added to an HF vessel. The mixture was treated with HF (10 mL) at -20°C and stirred. After 30 minutes, the
 10 HF was evaporated. The residue was suspended in 20% acetic acid and washed with diethyl ether (3X). The aqueous layer was lyophilized. The crude lyophilate was purified by reverse phase prep HPLC (0.1% trifluoroacetic acid in 0-40% aqueous acetonitrile, C-18 reverse-phase), and lyophilized to
 15 afford 14.4 g of the title compound in 26% yield. $t_R=12.5$ minutes (0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes) and MS ($M+H=564.5$).

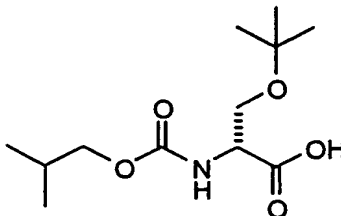
(iv) Examples 35 to 40 describe an alternative synthetic route to make Compound 1.



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Example 35Synthesis of i-butoxycarbonyl-D-Ser(O-t-Bu)-OMe

To a stirred homogeneous solution of HCl-D-Ser(O-t-Bu)-OMe (15 g , 71 mmol, Bachem) in tetrahydrofuran (200 mL), was added saturated sodium bicarbonate (80 mL), followed by isobutylchloroformate (19.45 g, 142 mmol). Layers were separated and the aqueous layer was washed with ethyl acetate (50 mL). The organic phases were combined and solvent was removed under reduced pressure. The residue was suspended in ethyl acetate (100 mL) and washed with 1M HCl (100 mL), saturated sodium bicarbonate (100 mL), and brine (100 mL). The organic layer was dried with magnesium sulfate, treated with decolorizing charcoal, (such as that sold under the trade name Darco), filtered, and solvent removed under reduced pressure, giving quantitative yield of the title compound. $R_f = 0.3$ (20% ethyl acetate/hexanes).

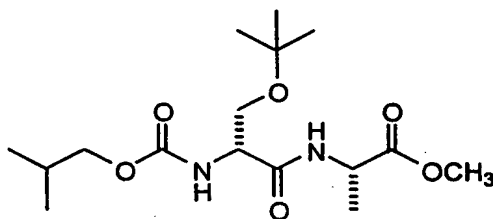
Example 36Synthesis of i-butoxycarbonyl-D-Ser(O-t-Bu)-OH

To a stirred solution of the compound of Example 35 (19.51 g, 70 mmol) in tetrahydrofuran (78 mL), was added lithium hydroxide (78 mmol, 3.28 g). The reaction mixture was

stirred vigorously for 3 hours until no starting material was observed by TLC (20% ethyl acetate/hexanes). The solution was acidified with concentrated HCl to pH~2 and the solvent removed under reduced pressure. The crude product was
5 suspended in ethyl acetate and extracted with saturated sodium bicarbonate (2X, 75 mL). The combined sodium bicarbonate washes were acidified with 6M HCl and the oil that separated was extracted with ethyl acetate (2 x 100 mL). The combined organic layers were dried with magnesium
10 sulfate, treated with Darco, filtered, and solvent removed under reduced pressure, giving quantitative yield of the title compound. R_f = 0.01 (20% ethyl acetate in hexanes).

Example 37

Synthesis of i-butoxycarbonyl-D-Ser(O-t-Bu)-Ala-OMe



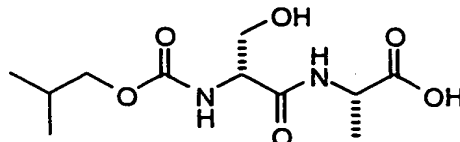
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To a solution of the compound of Example 36 (16.5 g, 63 mmol), HCl-Ala-OMe (10.6 g, 76 mmol), 1-hydroxybenzotriazole (10.2 g, 76 mmol), and EDC (16.33 g, 85 mmol) in acetonitrile (280 mL) at 0°C, was added 4-methylmorpholine (35 mL, 315
20 mmol). This mixture was stirred for 1 hour at 0°C, then at ambient temperature for 72 hours. The solvent was removed under reduced pressure and the resulting residue was suspended in ethyl acetate (300 mL) and 1M HCl (350 mL). The aqueous layer was separated and washed with ethyl acetate
25 (300 mL). The combined ethyl acetate layers were combined and washed with 1M HCl (300 mL), saturated sodium bicarbonate (400 mL), and brine (200 mL). The organic layer was dried

with magnesium sulfate, treated with Darco, filtered and solvent removed under reduced pressure, yielding 21.48 g of the title compound (98% yield).

Example 38

5 Synthesis of i-butoxycarbonyl-D-Ser-Ala-OH



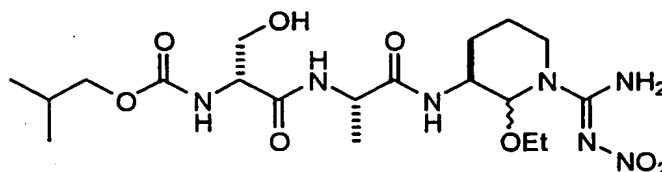
The compound of Example 37 (21 g, 58 mmol) was dissolved in trifluoroacetic acid (110 mL); the resulting mixture was stirred for 35 minutes. The solution was cooled in an ice bath, saturated sodium bicarbonate (630 mL) was added, followed by solid sodium bicarbonate (70 g) over 45 minutes to a resulting pH=7. The aqueous solution was extracted with ethyl acetate (3 x 250 mL). The combined organic extracts were combined, dried with magnesium sulfate, treated with Darco, filtered and the solvent removed in vacuo, giving a quantitative yield of i-butoxycarbonyl-D-Ser-Ala-OMe.

To a stirred solution of the crude residue in tetrahydrofuran (68 mL), was added lithium hydroxide (2.7 g, 64 mmol, 1.1 eq.) in water (17 mL). The reaction mixture was stirred vigorously for 0.5 hours until there was no more of the starting material observed by TLC (9:1 dichloromethane/isopropanol). The solution was acidified with 6M HCl (13 mL) to pH=2 and the solvent removed under reduced pressure. The crude product was suspended in ethyl acetate (400 mL) and water (50 mL). The aqueous layer was extracted with ethyl acetate (200 mL). The combined organic layers were dried with magnesium sulfate, filtered, and the solvent removed in vacuo, yielding 13.94 g of the title

compound (86% yield). $R_f = 0.3$ (90:30:5
chloroform/methanol/acetic acid).

Example 39

Synthesis of i-butoxycarbonyl-D-Ser-Ala-N⁹-nitroArg-O(ethyl
cyclo1)



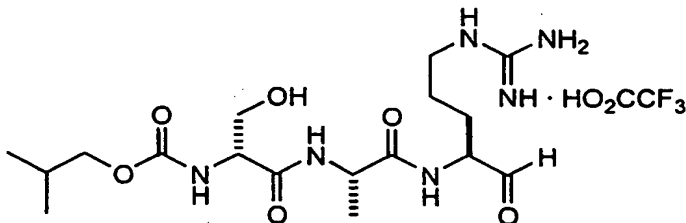
To mechanically stirred solution of 2,6-lutidine (30.5 mL, 272 mmol) in acetonitrile (520 mL) at 0°C, was added L-N⁹-nitroargininal ethyl cyclol, hydrochloride salt (16.44 g, 61 mmol). To this mixture, the compound of Example 38 (13.94 g, 50.5 mmol) in acetonitrile (200 mL) was added, followed by 1-hydroxybenzotriazole (8.32 g, 33 mmol). The resulting mixture was stirred rapidly. EDC (12.9 g, 67 mmol) was added in small portions over 15 minutes. The ice bath was removed, and the mixture was stirred for an additional 69 hours. The solution was concentrated under reduced pressure, and the residue dissolved in ethyl acetate (300 mL) and 6M HCl (200 mL). The resulting precipitate was filtered and dried to give Lot I. The layers from the filtrate were separated, and the organic layer washed with 1M HCl (200 mL). The combined aqueous layers were saturated with NaCl and extracted with ethyl acetate (300 mL). The combined organic layers were washed with saturated sodium bicarbonate (300 mL) and brine (150 mL), dried over magnesium sulfate, and the solvent removed under reduced pressure to give Lot II. The sodium bicarbonate washes were extracted with 20% isopropanol in dichloromethane (2 x 150 mL). The combined organic layers were dried with magnesium sulfate, and the solvent removed

100

under reduced pressure to give Lot III. Lots I, II, and III were each dissolved in minimum amount of hot acetonitrile and cooled overnight. The resulting solids were filtered and dried, yielding Lot I (4.15 g), Lot II (6.63 g), and Lot III (0.3 g). Lots I, II and III were identical by TLC and ^1H NMR. The combined yield of the title compound was 11.08 g, (45% yield).

Example 40

Synthesis of i-butoxycarbonyl-D-Ser-Ala-Arg-al,
10 trifluoroacetate salt (Compound 1)



To a solution of the compound of Example 39 (11.48 g, 23.5 mmol) in ethanol/acetic acid (230 mL of a 1:1 mixture), was added 10% palladium on carbon (5.74 g); the mixture was heated to 48°C. Over a period of 45 minutes, ammonium formate (8.82 g, 140 mmol,) in water (9 mL) was added. The mixture was stirred for 3 hours, cooled and filtered. The residue was washed with ethanol and solvent removed under reduced pressure. The residue was dissolved in water (250 mL) and lyophilized, giving quantitative yield of i-butoxycarbonyl-D-Ser-Ala-L-argininal ethyl cyclol. t_R =12 minutes at 0.1% trifluoroacetic acid in 15-25% aqueous acetonitrile over 20 minutes.

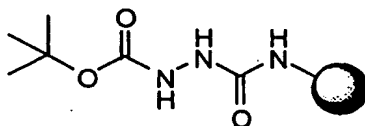
To i-butoxycarbonyl-D-Ser-Ala-L-argininal ethyl cyclol at 0°C, was added cold 6M HCl (115 mL). This mixture was stirred for 50 minutes. The ice bath was removed, and the reaction mixture was stirred at ambient temperature for 2

hours. After this time, the solution was recooled in ice bath, and sodium acetate (100 g) in water (200 mL) was added. The solution was filtered and purified by prep HPLC (using a 0.1% trifluoroacetic acid step gradient of 10,18,30,50% aqueous acetonitrile, C-18 reverse phase column) to afford three lots of the title compound: lot I (1.41 g, 14%, 99% purity), lot II (1.52 g, 16%, 98% purity), and lot III (3.13 g, 32%, 95% purity). t_R =9.46 minutes, 12.82 minutes and 13.87 minutes (reverse-phase HPLC, C-18, 0.1% trifluoroacetic acid in 10-20% aqueous acetonitrile over 20 minutes). MS (M+H=417.5).

(v) Examples 41 to 45 describe the synthesis of argininal-hydrazonylcarbonyl-aminomethylated polystyrene resin used to synthesize compounds of the present invention. See Figure 6.

Example 41

Preparation of t-Butoxycarbonyl-Hydrazonyl-Carbonyl-Aminomethylated Polystyrene Resin

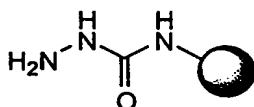


To a suspension of 1,1-carbonyldiimidazole (29.19 g, 180 mmol, 6 equiv.) in dimethylformamide (300 mL), was added portionwise t-butylcarbazate (23.76 g, 180 mmol, 6 equiv.) with stirring at ambient temperature under a nitrogen atmosphere. After the addition was completed, the reaction mixture was allowed to stir at room temperature for 2 ½ hours and then poured into aminomethylated polystyrene resin (30 g, 1 meq/g, Advance Chemtech) in the peptide synthesis flask. The suspension was purged with nitrogen gas for 3 hours. The resin was filtered and washed with 3 X 350 mL portions of

methylene chloride. Kaiser Test (solution and bead; light blue, clear). A double coupling was undertaken to ensure a quantitative reaction; however, double coupling is optional. Thus, a solution of 1,1-carbonyldiimidazole (14.60 g, 90 mmol, 3 equiv.) and t-butylcarbazate (11.88 g, 90 mmol, 3 equiv.) in 150 dimethylformamide was stirred at ambient temperature for 30 minutes and then poured into the previously prepared resin. After purging with nitrogen gas for 1 hour, the resin was filtered and washed with 6 X 350 mL portions each of methylene chloride, methanol, methylene chloride, methanol, methylene chloride, and methanol. Kaiser Test (solution and bead; clear and clear). The resin was dried under vacuum and acetylated with DMF/acetic anhydride/Et₃N (8:1:1; about 300 mL) for 30 minutes at ambient temperature. The resin was successively washed 3 times each with solvents (methylene chloride and methanol) and dried under vacuum to afford product in quantitative yield.

Example 42

20 Preparation of Hydrazoneylcarbonyl-Aminomethylated Polystyrene Resin



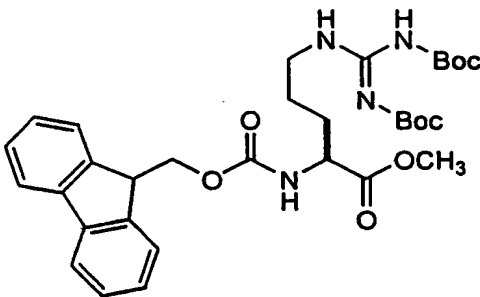
A solution of DCM/TFA (1:1) (300 mL) and thioanisole (10 mL) was added to the resin of Example 41 (30 g) to effect deprotection. The mixture was purged slowly with the nitrogen gas in the peptide synthesizer flask at ambient temperature for 30 minutes. The deprotected resin was filtered and washed with DCM (2X), DCM/DIEA (2X), DCM (2X) and alternatively with organic solvents (methylene chloride, methanol). The resulting title resin was dried under vacuum.

Kaiser Test (solution and bead; light blue, sand color); this synthesis afforded overall yield 30.64 g resin (approx. 0.85 meq semicarbazide/g).

Alternatively, the title resin was prepared as follows:
5 to amino methylated polystyrene resin (1.0 g, 1.0 mmol, Advanced Chemtech) in N,N-dimethylformamide (12 mL) was added 1,1-carbonyldiimidazole (3.89 g, 24.0 mmol, 24 equiv.). The suspension was shaken at room temperature for 5 hours and then filtered in a peptide synthesis flask. The resin was
10 washed with copious amounts of methylene chloride. Kaiser Test (solution and bead; light blue, white). A 2M solution of hydrazine in N,N-dimethyl formamide (12 mL) was added to the resin and allowed to shake at ambient temperature overnight. The resin was filtered and washed alternately
15 with organic solvents (methylene chloride and methanol) and dried under vacuum to afford title product 1.06 g (approx. 0.94 mmol/g).

Example 43

Preparation of N- α -fluorenylmethyloxycarbonyl-omega, omega'-
20 di-N-t-butoxycarbonyl-arginine methyl ester:

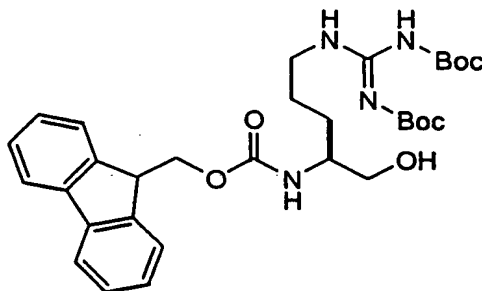


To a solution of N- α -fluorenylmethyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl-arginine (4.97 g, 8.329 mmol, Advanced Chemtech) in acetonitrile (82 mL), was added
25 potassium carbonate (1.38 g, 10 mmol) and methyl iodide (1.037 mL, 16.66 mmol). The mixture was warmed to 50°C.

After stirring for 4.5 hours at 50°C, the reaction mixture was poured into ethyl acetate (500 mL) and washed successively with water (1 x 50 mL), saturated sodium bicarbonate (1 x 50 mL), and brine (1 x 50 mL). The organic
5 phase was dried with sodium sulfate, and solvent was removed under reduced pressure yielding 4.7 g of the title compound.

Example 44

Preparation of N- α -fluorenylmethyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl-argininol



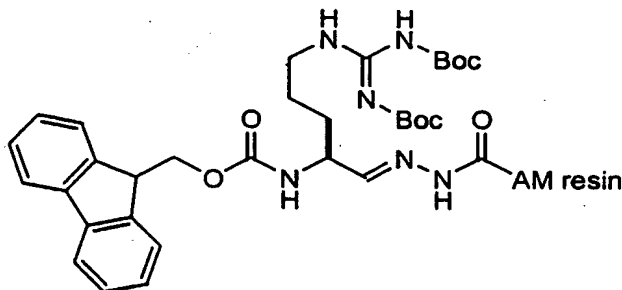
10

To a solution of the compound of Example 43 (5.675 g, 9.29 mmol) in tetrahydrofuran (15.5 mL) and methanol (93 mL), was added calcium chloride (2.06 g, 18.58 mmol). The mixture was cooled in an ice bath. Sodium borohydride (1.4 g, 37.17
15 mmol) was added slowly in portions to the stirred cooled solution. After 1.5 hours, solvent was removed under reduced pressure. The residue was suspended in ethyl acetate (500 mL) and water (300 mL). The phases were separated, and the organic layer was washed successively with 0.5M HCl (200 mL)
20 and brine (200 mL). The combined aqueous washes were back extracted with ethyl acetate. The ethyl acetate layers were combined, silica was added (30 g), and solvent was removed under reduced pressure. This silica was loaded onto a 150 x 80 mm silica flash column, and the product was eluted with
25 50% ethyl acetate/hexanes to afford 3.855 g (71% yield) of the title compound. 0.938 g (17% yield) of the compound of

Example 43 (starting material) was recovered. $R_f=0.30$ (50% ethyl acetate/hexanes). ^1H NMR (CDCl_3): 8.35ppm(bs, 1H), 7.75ppm(m, 2H), 7.6ppm(m, 2H), 7.38ppm(m, 2H), 7.3ppm(m, 2H), 5.45ppm(m, 1H), 4.4ppm(d, 2H), 4.2ppm(m, 1H), 3.7ppm(m, 2H), 3.57ppm(m, 1H), 3.45ppm(m, 1H), 3.35ppm(m, 2H), 2.15ppm(m, 1H), 1.6ppm(m, 4H), 1.45ppm(d, 18H).

Example 45

Preparation of N- α -fluorenylmethyloxycarbonyl-omega, omega'-di-N-t-butoxycarbonyl argininal hydrazonylcarbonyl aminomethylated polystyrene resin



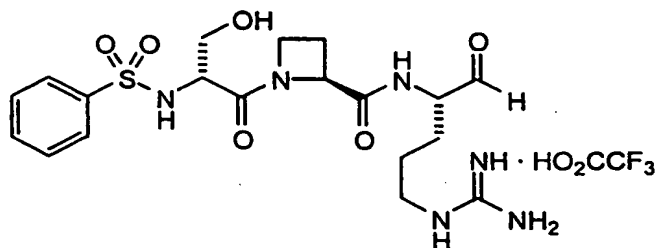
To a solution of the compound of Example 44 (3.62 g, 6.21 mmol) in methyl sulfoxide (31 mL) and toluene (31 mL) cooled in an ice bath, was added EDC (11.93 g, 62.1 mmol) and dichloroacetic acid (2.56 mL, 31.06 mmol). The reaction mixture was stirred for 40 minutes until no starting material was observed by TLC (50% ethyl acetate/hexanes). Water (150 mL) and ethyl acetate (500 mL) were added, and the phases were separated. The organic layer was then washed successively with 0.5M HCl (100 mL), saturated sodium bicarbonate (2 x 100 mL), and brine (100 mL). The organic layer was dried with sodium sulfate and solvent was removed under reduced pressure. This residue was dissolved in dichloromethane (45 mL), added to the compound of Example 42 (3.65 g, 3.1 mmol), and stirred in a sealed tube end over end overnight. The resin was filtered and washed successively

with dichloromethane (3 x 50 mL), dimethylformamide (3 x 50 mL), and methanol (3 x 50 mL), then dried under high vacuum. Yield of title compound was 4.3 g. The piperidine fulvene determination of the loading showed 0.37 mmol/g (60% coupling).

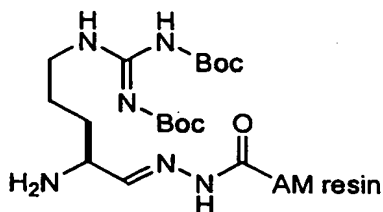
(vi) Example 46 describes the synthesis of benzenesulfonyl-D-seryl-azetidyl-argininal, trifluoroacetate salt (Compound 31). See Figure 7.

Example 46

10 Preparation of benzenesulfonyl-D-seryl-azetididyl-argininal, trifluoroacetate salt



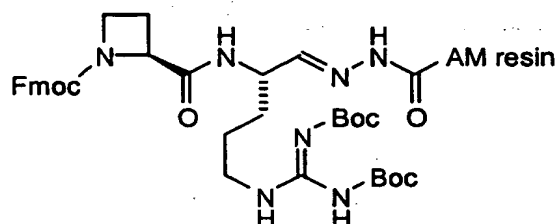
15 Step 1: Preparation of omega, omega'-di-N-t-butoxycarbonyl-argininal hydrazonylcarbonyl aminomethylated polystyrene resin



The resin product of Example 45, (125 mg) was treated with 30% piperidine/dimethylformamide (1.5 mL) in a 4 mL polypropylene column. After 30 minutes, the resin was drained, washed successively with dichloromethane (2 X 3 mL) and methanol (2 x 3 mL), and dried under vacuum to give the title compound. A ninhydrin assay on a small aliquot gave

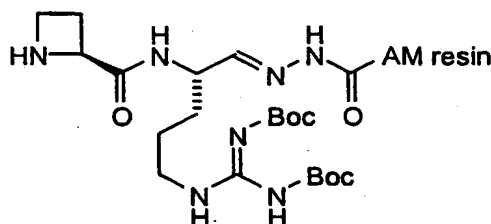
dark blue resin and solution showing a high yield for the deprotection.

Step 2: Preparation of N- α -Fmoc-azetidine-2-carbonyl-
omega, omega'-di-N-t-butoxycarbonyl-argininal
5 hydrazonylcarbonyl aminomethylated polystyrene resin:



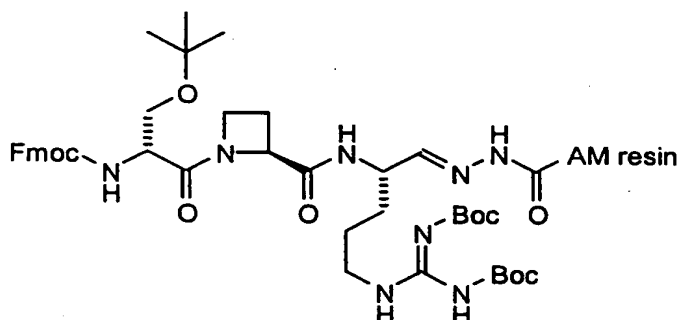
To the compound of Step 1 (0.12 g) in a 4 mL polypropylene column, were added N- α -Fmoc-azetidine-2-carboxylic acid (110 mg, 0.34 mmol), 1-hydroxybenzotriazole
10 (7.7 mg, .057 mmol), TBTU (37 mg, 0.11 mmol) and diisopropylethylamine (29.8 μ L, 0.17 mmol) in dimethylformamide (95 μ L). The reaction mixture was shaken at room temperature overnight. The reagents were drained from the resin and the resin was washed successively with
15 dichloromethane (2 X 3 mL) and methanol (2 X 3 mL). The resin was vacuum dried and a small aliquot was taken for ninhydrin colorimetric analysis which showed a 97% coupling efficiency in the production the title compound.

Step 3: Preparation of azetidine-2-carbonyl-omega,
20 omega'-di-N-t-butoxycarbonyl-argininal hydrazonylcarbonyl
aminomethylated polystyrene resin



The compound of Step 2 was treated with 30% piperidine/dimethylformamide (1.5 mL) in a 4 mL polypropylene column. After 30 minutes, the resin was drained, washed successively with dichloromethane (2 X 3 mL) and methanol (2 x 3 mL), and dried under vacuum to give the title compound. A ninhydrin assay on a small aliquot gave dark blue resin and solution showing a high yield for the deprotection.

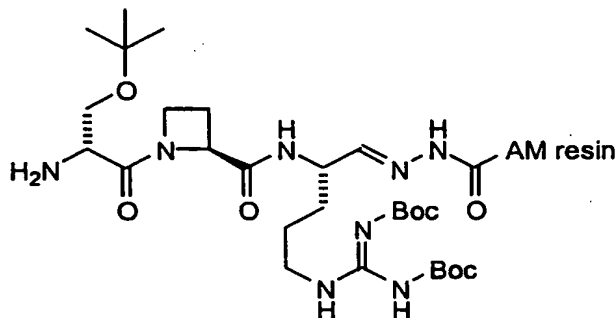
Step 4: Preparation of N- α -Fmoc-seryl(O-t-Bu)-azetidine-2-carbonyl-N-omega, omega'-di-N-t-butoxycarbonyl-argininal hydrazonylcarbonyl aminomethylated polystyrene resin:



To the compound of Step 3 (0.12 g) in a 4 mL polypropylene column, were added N- α -Fmoc-D-serine t-butyl ether (43 mg, 0.11 mmol), 1-hydroxybenzotriazole (7.7 mg, 0.057 mmol), TBTU (37 mg, 0.11 mmol) and diisopropylethylamine (29.8 μ L, 0.17 mmol) in dimethylformamide (95 μ L). The reaction mixture was shaken 4 hours at room temperature. The reagents were drained from the resin; the resin was washed successively with dichloromethane (2 X 3 mL), and methanol (2 X 3 mL). The resin was dried under vacuum, then double coupled using N- α -Fmoc-serine O-t-butyl ether (43 mg, 0.11 mmol), 1-hydroxybenzotriazole (7.7 mg, .057 mmol), TBTU (37 mg, 0.11 mmol) and diisopropylethylamine (29.8 μ L, 0.17 mmol) in dimethylformamide (95 μ L). The reaction mixture was

shaken 2 hours at room temperature. The reagents were drained from the resin, then the resin was washed successively with dichloromethane (2 X 3 mL) and methanol (2 X 3 mL). The resin was dried under vacuum, and a small aliquot was taken for ninhydrin colorimetric analysis which showed a 97.4% coupling efficiency in the production the title compound.

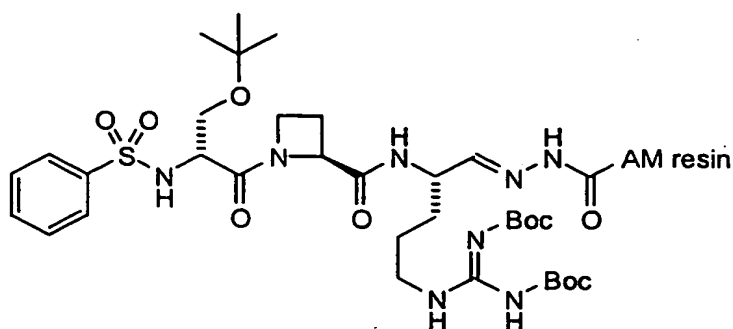
Step 5: Preparation of seryl(O-t-Bu)-azetidine-2-carbonyl-omega, omega'-di-N-t-butoxycarbonyl-argininal hydrazonylcarbonyl aminomethylated polystyrene resin



The compound of Step 4 was treated with 30% piperidine/dimethylformamide (1.5 mL) in a 4 mL polypropylene column. After 30 minutes, the resin was drained, washed successively with dichloromethane (2 X 3 mL) and methanol (2 x 3 mL), and dried under vacuum to give the title compound. A ninhydrin assay on a small aliquot gave dark blue resin and solution showing a high yield for the deprotection.

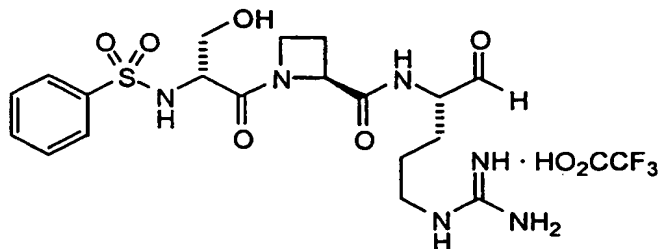
Step 6: Preparation of N-α-benzenesulfonyl-D-seryl(O-t-Bu)-azetidine-2-carbonyl-omega, omega'-di-N-t-butoxycarbonyl-argininal hydrazonylcarbonyl aminomethylated polystyrene resin

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To the compound of Step 5 (0.12 g) in a 4 mL polypropylene column, were added benzenesulfonyl chloride (17 μ L, 0.133 mmol), and diisopropylethylamine (46.3 μ L, 0.27 mmol) in dimethylformamide (888 μ L). The reaction mixture was shaken overnight at room temperature. The reagents were drained from the resin; the resin was washed successively with dichloromethane (2 X 3 mL) and methanol (2 X 3 mL). The resin was dried under vacuum, then double coupled with benzenesulfonyl chloride (17 μ L, 0.133 mmol) and diisopropylethylamine (46.3 μ L, 0.27 mmol) in dimethylformamide (888 μ L). The reaction mixture was shaken 3 hours at room temperature. The reagents were drained from the resin, and the resin was washed successively with dichloromethane (2 X 3 mL), and methanol (2 X 3 mL). The resin was dried under vacuum, and a small aliquot was taken for ninhydrin colorimetric analysis which showed a 96% coupling efficiency in the production the title compound.

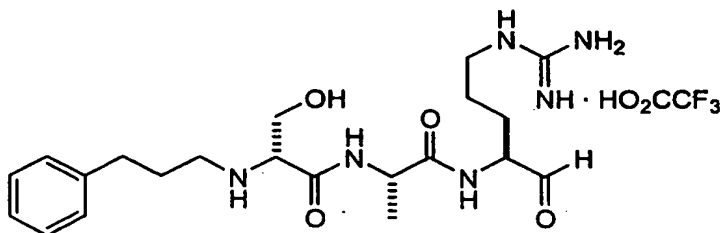
Step 7: Preparation of benzenesulfonyl-D-seryl-azetidine-2-carboxyl-argininal, trifluoroacetate salt



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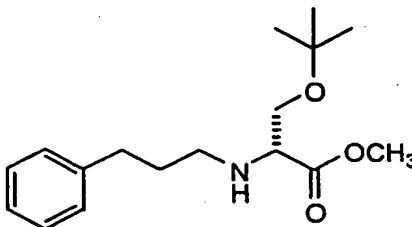
The compound of Step 6 (45 mg) in a 4 mL polypropylene fritted column was treated with trifluoroacetic acid/water (0.5 mL of a 9:1 mixture). The column was shaken for 1.5 hours at room temperature. The reaction solution was drained into a test tube and the resin was washed with water to a total volume of 5.2 mL filtrate. The title compound was purified by semi-preparative reverse-phase HPLC (0.1% trifluoroacetic acid in 0-40% aqueous acetonitrile, C-18 reverse-phase), and lyophilized to afford 14.4 g of the title compound in 26% yield. The pure fractions, as analyzed by HPLC, were combined and lyophilized to afford the title compound (2.1 mg). MS (M+H=469).

(vii) Examples 47 to 50 describe the synthesis of N- α -(3-phenylpropyl)-D-seryl-alanyl-argininal, trifluoroacetate salt (Compound 32).



Example 47

Preparation of N- α -(3-phenylpropyl)-D-serine-t-butyl ether methyl ester

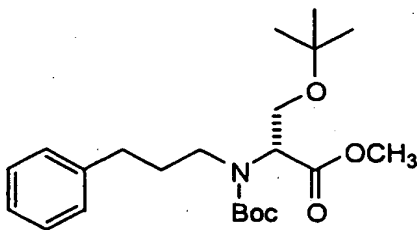


Serine O-t-butyl ether methyl ester (1.50 g, 7.1 mmol), hydrocinnamaldehyde (1.40 mL, 10.6 mmol), and triethylamine

(1.18 mL, 8.5 mmol) were refluxed in tetrahydrofuran (70 mL) for 4 hours. After the solution was allowed to cool to room temperature, sodium borohydride (0.46 g, 12 mmol) was added in two portions to the stirred solution. The reaction mixture was stirred at ambient temperature for 30 minutes; the solution was concentrated under reduced pressure. The residue was partitioned between ethyl acetate 1.0M HCl. The organic layer was washed with 1.0N HCl. The aqueous layer was basified with 40% NaOH to pH 10, then extracted with ethyl acetate (2X). The combined organic layers were dried over sodium sulfate; the solvent was removed in vacuo. The residue was purified by flash chromatography over silica gel (1 x 6 inch column) eluting with 10-30% ethyl acetate/hexanes to afford 150 mg (7% yield) of the title compound. $R_f = 0.60$ (50% ethyl acetate/hexanes).

Example 48

Preparation of N- α -t-butoxycarbonyl-N- α -(3-phenylpropyl)-D-serine-t-butyl ether methyl ester

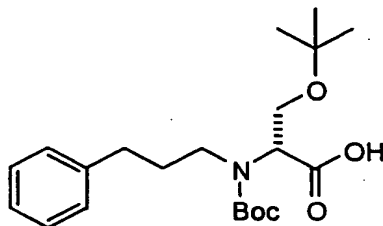


The compound of Example 47 (150 mg, 0.51 mmol), di-t-butyldicarbonate (167 mg, 0.77 mmol) and diisopropylethylamine (0.13 mL, 0.77 mmol) were stirred in tetrahydrofuran (2 mL) at ambient temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate (20 mL), washed successively with 1.0N HCl (2X), saturated sodium bicarbonate (2X), and brine (1X). The solvent was removed in vacuo to

afford 206 mg of the title compound in quantitative yield. R_f = 0.74 (50% ethyl acetate/hexanes).

Example 49

Preparation of N- α -t-butoxycarbonyl-N- α -(3-phenylpropyl)-D-serine-t-butyl ether



To a solution of the compound of Example 48 (206 mg, 0.52 mmol) in methanol (3.5 mL), was added dropwise 1.0N LiOH (0.63 mL, 0.63 mmol). The solution became cloudy, then
10 became homogeneous in 5 minutes. The reaction mixture was allowed to stir at ambient temperature overnight. Additional 1.0N LiOH (1.47 mL) was added. After 2 hours, additional 1.0N LiOH (1.0 mL) was added. After no starting material was observed by TLC (50% ethyl acetate/hexanes), the reaction
15 mixture was acidified to pH4 with DOWEX (50 X 8-400) ion exchange resin. The solution was filtered, rinsing with methanol, then water. The solution was concentrated under reduced pressure, then lyophilized to afford 189 mg of the title compound in 95% yield as a yellow oil. R_f = 0.04 (50%
20 ethyl acetate/hexanes).

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48	3-phenylpropylsulfonyl-D-seryl-alanyl-argininal	3-phenylpropylsulfonyl-D-serine (O-t-butyl)
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For synthesis of 2-phenylethylsulfonyl-D-serine-O-t-butyl, the procedures of Examples 22 and 23 were followed with two substitutions in Example 22. Either 2-phenylethylsulfonyl chloride or 3-phenylpropylsulfonyl chloride was substituted for α -toluenesulfonyl chloride, and 4-methylmorpholine was used in place of diisopropylethylamine.

Example 5210 Solid phase synthesis of Compounds 45, 46, and 49 to 51

Steps 1 through 4 and Step 7 of Example 46 are followed, with the substitution of Cbz-D-serine(O-t-butyl) for Fmoc-D-serine(O-t-butyl) in Step 4, and the noted substitution in Step 2 for Fmoc-L-azetidine-2-carboxylic acid:

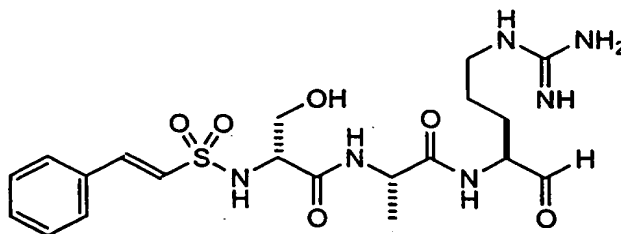
15

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 2
45	benzyloxycarbonyl-D-seryl-L-seryl-argininal	Fmoc-L-serine-O-t-butyl
46	benzyloxycarbonyl-D-seryl-L- β -cyanoalanine-argininal	Fmoc-L- β -cyanoalanine
49	benzyloxycarbonyl-D-seryl-L-cis-4-hydroxyprolyl-argininal	Fmoc-cis-4-hydroxyproline
50	benzyloxycarbonyl-D-seryl-L-3,4-dehydroprolyl-argininal	Fmoc-L-3,4-dehydroproline

COMPOUND NUMBER	COMPOUND NAME	SUBSTITUTION IN STEP 2
51	benzyloxycarbonyl-D-seryl-L-propargylglycyl-argininal	Fmoc-L-propargylglycine
52	benzyloxycarbonyl-D-seryl-L-allylglycyl-argininal	Fmoc-L-allylglycine

Example 53

Solid phase synthesis of trans- β -styrenesulfonyl-D-seryl-L-alanyl-argininal (Compound 53)

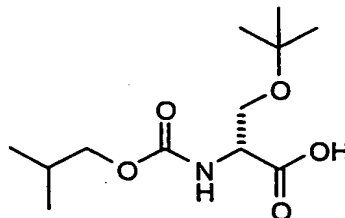


5 Steps 1 through 7 of Example 46 are followed, with the substitution of Fmoc-L-alanine for N α Fmoc-azetidine-2-carboxylic acid in Step 2 and the substitution of trans- β -styrenesulfonyl chloride for benzenesulfonyl chloride in Step 6, to make the title compound.

10 (ix) Examples 54 to 59 describe an alternate synthesis method for Compound 1. See Figure 5.

Example 54

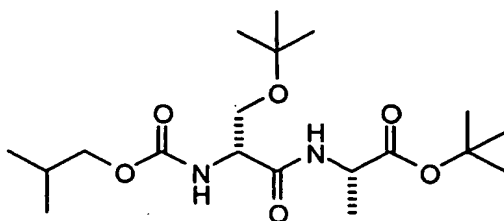
Preparation of i-butoxycarbonyl-D-Ser(O-t-butyl)-OH



To a solution of D-serine-O-t-butyl ether (10.5 g, 65.3 mmol) in water (51 mL) was added sodium carbonate (20.1 g, 196.2 mmol) followed by isobutyl chloroformate (9.8 mL, 75.2 mmol). After 3 hours this mixture became cloudy and was stirred for an additional 3 hours. After 6 hours the solution was acidified with 6M HCl (~50 mL). The reaction mixture was then extracted with ethyl acetate (3 x 200 mL). After the initial extraction, the aqueous layer was saturated with sodium chloride and extracted with ethyl acetate (2 x 200 mL). The ethyl acetate layers were combined and dried with sodium sulfate, followed by removal of the solvent in vacuo, yielding 17.0 g (99.5% yield) of the title compound as a white solid. HPLC: t=18.5 minutes in a 5% to 75% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 mL/min flow rate. NMR(CDCl₃): 5.5ppm(m,1H), 4.45ppm(bs,1H), 3.82-3.95ppm(m,3H), 3.52-3.6ppm(m,1H), 1.85-1.97ppm(m,1H), 1.2ppm(s,9H), 0.9ppm(d,6H).

Example 55

20 Preparation of i-butoxycarbonyl-D-Ser(t-Bu)-L-Ala-O-t-Bu

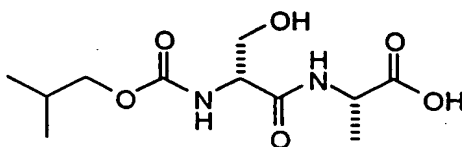


A solution of the compound of Example 54 (10 g, 38.3 mmol), L-alanine t-butyl ester, HCl salt (10.43 g, 57.4 mmol), EDC (11.05 g, 57 mmol), and hydroxybenzotriazole (5.85 g, 38.3 mmol) in acetonitrile (153 mL) was stirred for 15 minutes at room temperature. Diisopropylethylamine (32.7 mL, 191 mmol) was added and the reaction mixture stirred for 18

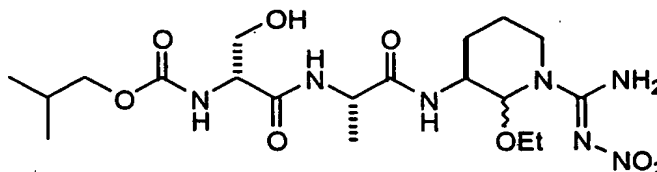
hours. The solvent was removed under reduced pressure; the resulting residue was resuspended in ethyl acetate (1000 mL) and 1M HCl (100 mL). The ethyl acetate layer was washed with 0.5M HCl (100 mL), saturated with sodium bicarbonate (2 x 100 mL), and brine (100 mL). The ethyl acetate layer was dried with sodium sulfate and solvent was removed in vacuo, resulting in a quantitative yield of title compound. HPLC: t_r =18.7 minutes in a 5% to 90% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate. NMR(CDCl₃): 7.15ppm(bs, 1H), 5.6ppm(bs, 1H), 4.4-4.5ppm(m, 1H), 4.2ppm(bs, 1H), 3.87-3.95ppm(m, 3H), 3.3-3.4ppm(m, 1H), 1.85-1.95ppm(m, 1H), 1.45ppm(s, 9H), 1.39ppm(d, 3H), 1.2ppm(s, 9H), 0.9ppm(d, 6H).

15 Example 56

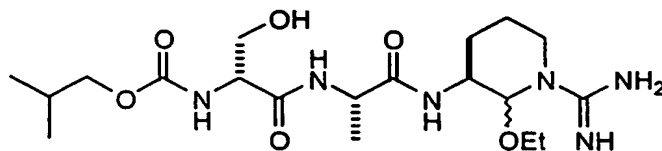
Preparation of i-butoxycarbonyl-D-Ser-L-Ala-OH



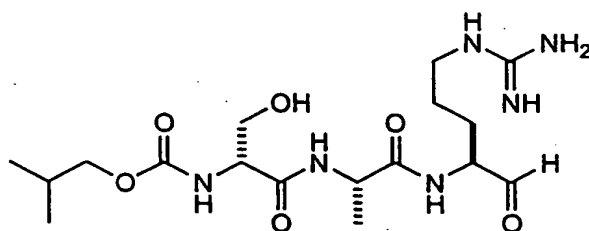
To a solution of the compound of Example 55 (7.15 g, 18.4 mmol) in dichloromethane (35 mL), was added trifluoroacetic acid (35 mL). This mixture was stirred for two hours, then solvent was removed under reduced pressure. Toluene was added and the solvents were removed in vacuo to remove the trifluoroacetic acid. A quantitative yield of the title compound as a viscous yellow oil was then carried on to the next step. t_r =10.5 minutes in a 5% to 90% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate.

Example 57Preparation of i-butoxycarbonyl-D-Ser-L-Ala-N⁹-nitroargininal-ethyl cyclol

5 A solution of the compound of Example 56 (5.09 g, 18.4 mmol), N⁹-nitroargininal ethyl cyclol, HCl salt (6.4 g, 23.9 mmol), EDC (5.31 g, 27.6 mmol), and hydroxybenzotriazole (2.82 g, 18.4 mmol) in acetonitrile (74 mL) was stirred for 15 minutes at ambient temperature. Diisopropylethylamine
10 (15.7 mL, 92 mmol) was added and the reaction mixture was stirred for 18 hours. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethyl acetate (1000 mL) and 0.5M HCl (100 mL). The ethyl acetate layer was washed with 0.5M HCl (100 mL), saturated sodium
15 bicarbonate (2 x 100 mL), and brine (100 mL), then dried with sodium sulfate. The solvent was removed under reduced pressure to a volume of ~25 ml resulting in a white precipitate. The reaction mixture was then left overnight at 4°C. The precipitate was then filtered and dried, resulting
20 in 4.6 g (50% yield) of title compound as a single peak by HPLC: t=11.5 minutes in a 5% to 90% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate.

Example 58Preparation of i-butoxycarbonyl-D-Ser-L-Ala-Argininal-ethyl cyclol

- 5 A solution of the compound of Example 57 (4.55 g, 9.3 mmol) in ethanol:water:acetic acid (55 mL of a 4:1:1 mixture) was flushed with nitrogen before palladium on carbon (1.15 g, 10% palladium, 50% water) was added. This mixture was hydrogenated at 50 psi for 18 hours. The reaction mixture
- 10 was then filtered to remove palladium and solvent was removed in vacuo to give a quantitative yield of the title compound. HPLC: t_r =14.2 minutes in a 5% to 50% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate.

15 Example 59Preparation of i-butoxycarbonyl-D-Ser-L-Ala-Argininal (Compound 1)

- A solution of the compound of Example 58 (9.3 mmol) in 20 3M HCl was allowed to stir for three hours. This material was then loaded directly onto a 50 x 300 mm, 15 micron particle, 100 angstrom pore, C18 column at a 80 mL/minute flow rate in three lots. The fractions containing product were pooled and lyophilized, yielding 2.38 g (61% yield) of
- 25 the title compound that was 97% pure by analytical HPLC.

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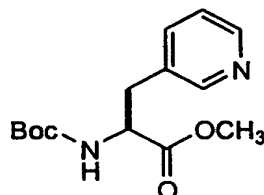
HPLC: t=16.4 minutes, 18.9 minutes and 19.6 minutes in a 5% to 25% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 micron particle, 100 angstrom pore, C18 column at a 1 mL/min flow rate. Mass spec (M+H) = 417.

5 NMR(D₂O): 5.4ppm(s, 1H), 4.25-4.37ppm(m, 1H), 4.1-4.25ppm(m, 1H), 3.75-3.95ppm(m, 5H), 3.42-3.52ppm(m, 1H), 3.2-3.3ppm(m, 1H), 1.58-1.98ppm(m, 5H), 1.35ppm(d, 3H), 0.85ppm(d, 6H).

(x) Examples 60 to 72 describe synthesis of certain intermediates which may be used in synthesis of compounds of the present invention. See Figure 8 (Examples 60 to 64) and Figure 9 (Examples 65 to 72).

Example 60

Preparation of N-(t-butoxycarbonyl)-3-(3-pyridyl)-L-alanine methyl ester



15

To a solution of N-(t-butoxycarbonyl)-3-(3-pyridyl)alanine (5.0 g, 18.8 mmol) in methanol (100 mL), was added thionyl chloride (2M solution in dichloromethane, 66 mL, 132 mmol). The resulting solution was stirred overnight at ambient temperature. The methanol was removed under reduced pressure to a minimum volume and ethyl acetate (100 mL) was added. The resulting white precipitate was collected in a fritted funnel. To a solution of the collected precipitate in a mixture of tetrahydrofuran/water (40 mL each), was added di-tert-butyl dicarbonate (4.8 g, 21.99 mmol) and sodium carbonate (1.95 g, 18.4 mmol). After stirring for 12 hours at ambient temperature, the reaction mixture was diluted with ethyl acetate (40 mL) and washed

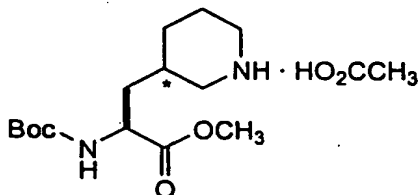
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with a solution of saturated sodium bicarbonate (25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum to give crude product. This product was subjected to flash column chromatography on silica gel (230-400 mesh) using a 8 x 52 cm column and eluting with a 10:90 mixture of ethyl acetate/hexane, followed by a 60:40 mixture of ethyl acetate/hexane. 4 g (74%) of the title compound was obtained as an oil. Thin-layer chromatography (silica gel; ethyl acetate), $R_f = 0.68$.

10 Example 61

Preparation of N-(t-butoxycarbonyl)-3-([R,S]-3-piperidyl)-L-alanine methyl ester, acetate salt

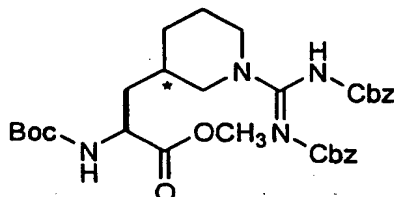


A solution of the compound of Example 60 (5 g, 17.8 mmol) in ethanol (24 mL), acetic acid (6 mL) and water (6 mL) was hydrogenated over platinum oxide (500 mg) at 45 psi for three hours. The catalyst was filtered off and the filtrate was concentrated under vacuum to an oily residue (6.89 g) which was used in the procedure described in Example 62 without further purification. Thin-layer chromatography yielded two spots corresponding to two diastereomers with R_f values of 0.16 and 0.26, respectively (silica gel; 4:1:1 n-butanol/ acetic acid/water).

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Example 62

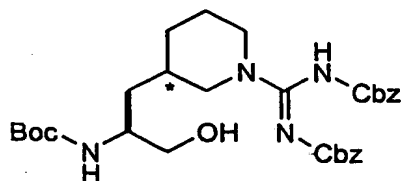
Preparation of N-(t-butoxycarbonyl)-3-[(R,S)-3-piperidyl-(N-guanidino (bis-benzyloxycarbonyl))]-L-alanine methyl ester



5 To a solution of the compound of Example 61 (6.89 g, 19.9 mmol) in tetrahydrofuran (80 mL), was added S-methylisothiourea bis-benzyloxycarbonyl (7.13 g, 19.9 mmol), followed by N-methylmorpholine (4.37 mL). The reaction mixture was stirred at ambient temperature for 18 hours. The
10 reaction mixture then was concentrated under vacuum and the resulting residue was dissolved in ethyl acetate (100 mL) and washed with 1N sodium bisulfate and saturated sodium chloride (50 mL each). After drying over anhydrous sodium sulfate, the solvents were removed under vacuum; the crude title
15 compound was subjected to flash column chromatography on silica gel (230-400 mesh) using a 8x52 cm column and eluting with 1:9 ethyl acetate/hexanes (two column volumes) followed by 1:1 ethyl acetate/hexanes. 2.75 g the title compound was obtained as a mixture of two diastereomers. Thin-layer
20 chromatography gave two spots with R_f values of 0.57 and 0.62, respectively (silica gel; 1:1 ethyl acetate/ hexanes).

Example 63

Preparation of N-(t-butoxycarbonyl)-3-[(R,S)-3-piperidyl-(N-guanidino (bis-benzyloxycarbonyl))]-L-alaninol

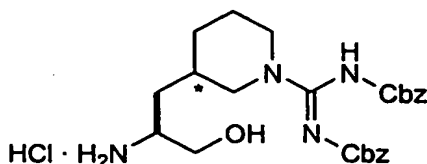


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To a stirred solution of the compound of Example 62 (2.23 g, 3.7 mmol) in absolute ethanol (8 mL) and anhydrous tetrahydrofuran (4 mL), was added calcium chloride (844 mg, 7.6 mmol) and sodium borohydride (575 mg, 15.2 mmol). After stirring 12 hours at ambient temperature, the reaction mixture was concentrated under vacuum and the resulting residue was partitioned between ethyl acetate and 1N sodium bisulfate (10 mL each). The two layers were separated; the organic layer was washed twice more with 1N sodium bisulfate, dried over anhydrous sodium sulfate and concentrated under vacuum to give a residue. Flash column chromatography of the residue on silica gel (230-400 mesh) using a 5.5x45 cm column and eluting with ethyl acetate gave 1.3 g of the title compound as a white foam. Thin layer chromatography yielded two spots corresponding to two diastereomers with R_f values of 0.18 and 0.27, respectively (silica gel; 1:1 ethyl acetate/ hexanes).

Example 64

Preparation of 3-[(R,S)-3-piperidyl-(N-guanidino(bis-benzyloxycarbonyl))]-L-alaninol, hydrochloride salt



The compound of Example 63 (290 mg, 0.57 mmol) was treated with 2.5N anhydrous hydrochloric acid in ethyl acetate (2.0 mL) at ambient temperature for one hour. The solvent was removed under vacuum to a sticky-white solid (260 mg). This solid is used without further isolation to prepare a compound of the present invention having a 3-piperidinyl-

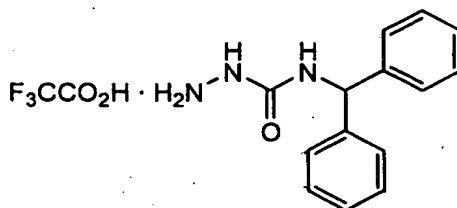
125

(N-guanidino)-alaninal at P1. ^1H NMR spectrum taken in CD_3OD showed no t-butoxycarbonyl protons at 1.4 ppm.

Example 65

Preparation semicarbazid-4-yl diphenylmethane,

5 trifluoroacetate salt



Step 1:

A solution of carbonyldiimidazole (16.2 g, 0.10 mole) in 225 mL of dimethylformamide was prepared at room temperature and allowed to stir under nitrogen. A solution of t-butyl carbazate (13.2 g, 0.100 mole) in 225 mL dimethylformamide was then added dropwise over a 30 minute period. Next, diphenylmethylaniline (18.3 g, 0.10 mole) was added over a 30 minute period. The reaction mixture was allowed to stir at room temperature under nitrogen for one hour. Water (10 mL) was added and this mixture was concentrated to about 150 mL under vacuum. This solution was poured into 500 mL water and then extracted with 400 mL of ethyl acetate. The ethyl acetate phase was extracted two times each with 75 mL 1N HCl, water, saturated sodium bicarbonate and brine, and then was dried with anhydrous magnesium sulfate. The mixture was filtered and the solution was concentrated to give 29.5 g (85% yield) of 1-t-butoxycarbonyl-semicarbazid-4-yl diphenylmethane as a white foam. This material may be purified by recrystallization from ethyl acetate/hexane, but was pure enough to use directly in step 2: mp 142-143°C. ^1H NMR (CDCl_3) delta 1.45 (s, 9H), 6.10 (dd, 2H), 6.42 (s, 1H), 6.67 (bs, 1H), 7.21-7.31 (m, 10H). Analysis calculated for

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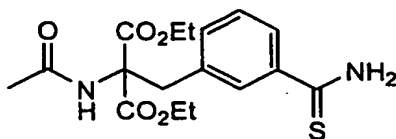
C₁₉H₂₃N₃O₃: C, 66.84; H, 6.79; N, 12.31. Found: C, 66.46; H, 6.75; N; 12.90.

Step 2:

A solution of 3.43 g (10 mmol) of 1-t-butoxycarbonyl-
5 semicarbazid-4-yl diphenylmethane in 12.5 mL of
dichloromethane was treated with 12.5 mL of trifluoroacetic
acid at 0°C. The reaction mixture was allowed to stir for 30
minutes at this temperature. The reaction mixture was then
added dropwise to 75 mL of diethyl ether to give a
10 precipitate. The resulting precipitate was filtered off and
washed with diethyl ether to give 2.7 g (80% yield) of the
title compound; mp 182-184°C.

Example 66

Preparation of 3-thioamidobenzyl-N-acetylaminomalonate
15 diethyl ester



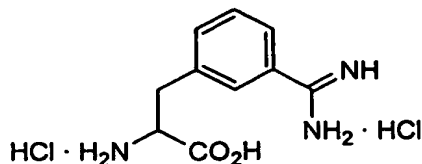
To a stirred solution of α -bromo-meta-tolunitrile (45.0
g, 0.24 mole), diethyl acetamidomalonate (48.0 g, 0.22 mole)
and potassium iodide (3.0 g, 0.018 mole) in dioxane (500 mL),
20 was added 2.5M sodium ethoxide in ethanol (100 mL) dropwise
under an argon atmosphere. After the addition was complete,
the solution was refluxed for 6 hours. The reaction mixture
was allowed to stand overnight at room temperature, then
diluted with brine (250 mL) and water (250 mL), and extracted
25 with ethyl acetate four times (1.0 L total). The combined
extracts were washed with water (100 mL), 10% citric acid
(100 mL), water (100 mL) and brine (2 x 50 mL), then dried
over anhydrous magnesium sulfate and filtered. The solvent

was removed under vacuum. The crude residue was recrystallized from ethyl acetate and diethyl ether in two crops to yield 43.51 g (60%) of the 3-cyanobenzyl-N-acetylamino malonic acid diethyl ester as yellow crystals.

- 5 $\text{H}_2\text{S}(\text{g})$ was bubbled into a rapidly stirring solution of 3-cyanobenzyl-N-acetylamino malonic acid diethyl ester (44.3 g, 0.13 mmol) in pyridine (300 mL) and triethylamine (100 mL) for 40 minutes. The reaction mixture was stirred at room temperature for 16 hours, then poured into 3.0 L of water. A
10 yellow precipitate formed immediately. The solution was allowed to stand at 4°C for 4 hours, then was filtered. The crude title compound was recrystallized from ethyl acetate and hexanes to yield 48.1 g (98% yield) of the title compound as yellow crystals, m.p. 183-186°C. ^1H NMR (CDCl_3): δ 1.31 (t, $J=7.1$ Hz, 6H), 2.06 (s, 3H), 3.70 (s, 2H), 4.29
15 (q, $J=7.1$ Hz, 4H), 4.80-4.87 (m, 1H), 6.60 (s, 1H), 7.10-7.20 (m, 1H), 7.27-7.35 (m, 2H), 7.60-7.70 (m, 2H). Analysis calculated for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_5\text{S}$: C, 55.72; H, 6.05; N, 7.64. Found: C, 55.55; H, 5.96; N, 7.76.

20 Example 67

Preparation of 3-amidino-D,L-phenylalanine, dihydrochloride salt



- The compound of Example 66 (48.1 g, 0.13 mmol) was
25 dissolved in acetone (800 mL). Iodomethane (18.3 mL, 0.19 mole, 1.5 equivalents) was added and the solution was refluxed for 30 minutes. The solution was cooled to room temperature; the intermediate thioimide was filtered, dried

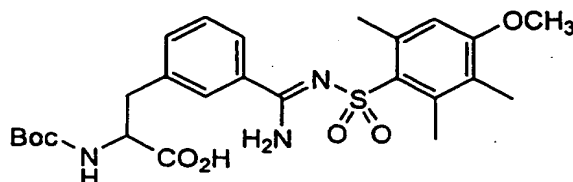
128

and dissolved in methanol (500 mL). Ammonium acetate (14.8 g, 0.19 mole, 1.5 equivalents) was added. The reaction mixture was refluxed for 1 hour, then cooled to room temperature and poured into ether (1.2 L). The solution was allowed to stand at 4°C for 72 hours. The crude 3-amidinobenzyl-N-acetylaminomalononic acid diethyl ester was filtered, washed with ether, air dried, and then refluxed in concentrated HCl (250 mL) for 3 hours. The reaction mixture was concentrated under vacuum, diluted with water (0.5 L), and concentrated under vacuum again. These steps were repeated. The crude title compound was purified by cation-exchange (Sephadex SP-C25) using a gradient of 0-1.0N HCl as eluent to yield 10.8g (30% yield) of the title compound as an off-white solid. ¹H NMR (D₂O): delta 3.14-3.29 (2H, m), 4.17 (dd, J=7.4, 6.2 Hz, 1H), 7.42-7.69 (4H, m). Analysis calculated for

C₁₀H₁₃N₃O₂·2HCl·1.9H₂O: C, 38.20; H, 6.03; N, 13.36.
Found: C, 38.51; H, 5.64; N, 12.89.

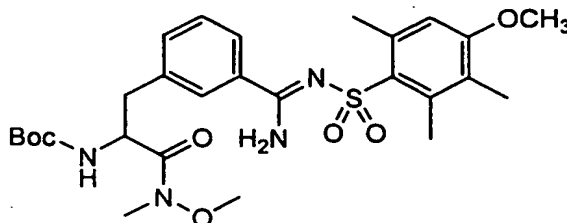
Example 68

20 Preparation of N-α-Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-3-amidino-D,L-phenylalanine

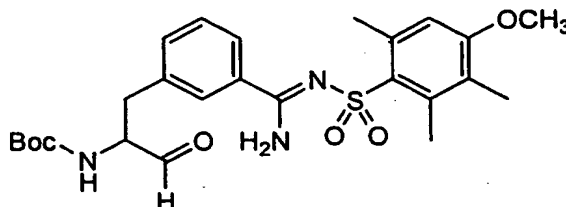


3-amidino-D,L-phenylalanine (the compound of Example 67,) (4.00 g, 13 mmol) was dissolved in 50% aqueous dioxane (20 mL). Sodium bicarbonate (3.38 g, 40 mmol) was added, followed by di-t-butyl dicarbonate (2.93 g, 13 mmol) in dioxane (4 mL). The reaction mixture was stirred for 18 hours at room temperature. The solution was cooled in an ice

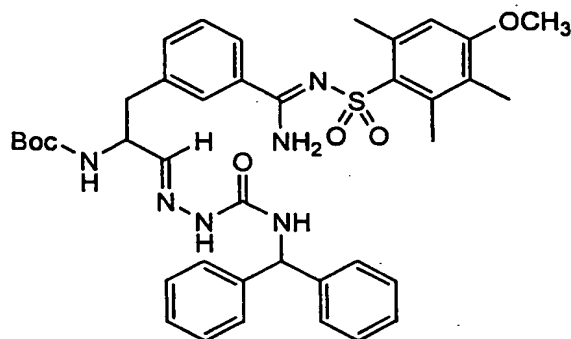
bath, then 4.0 N sodium hydroxide was added dropwise until the solution was pH 12. 4-methoxy-2,3,6-trimethylbenzenesulfonyl chloride (8.01 g, 32 mmol) in dioxane (10 mL) was added dropwise. 4.0 N sodium hydroxide was added as needed to keep the pH at 12. The ice bath was removed. After 1 hour, 1.0 N HCl was added to bring the solution to pH 7-8. The solution was diluted with an additional 50 mL of water and then was washed with ethyl acetate two times (20 mL each). The aqueous layer was acidified to pH 1.0 with 1.0 N HCl and extracted with ethyl acetate three times (100 mL total). The combined organic layers were washed with water (20 mL) and brine twice (10 mL each). The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. The residue was dissolved in a minimum amount of dichloromethane, then added dropwise to ether (25 mL). Solid impurities were removed by filtering and the solvent removed from the filtrate under vacuum to give 4.90 g (68% crude yield) of the title compound as an off-white foam. A 30 mg sample of the title compound was further purified by preparative thin-layer chromatograph developing with 1% acetic acid/5% isopropanol/dichloromethane to give 9 mg of the title compound in a purer form. $R_f = 0.16$ (1% acetic acid/5% isopropanol/dichloromethane). $^1\text{H NMR}$ (CD_3OD): δ 1.32 (s, 9H), 2.14 (s, 3H), 2.63 (s, 3H), 2.71 (s, 3H), 2.93 (dd, $J=13.7, 9.3$ Hz, 1H), 3.22 (dd, $J=13.7, 4.3$ Hz, 1H), 3.85 (s, 3H), 4.34-4.37 (m, 1H), 6.72 (s, 1H), 7.35-7.47 (2H, m), 7.69-7.75 (m, 2H).

Example 69Preparation of N- α -Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-3-amidino-D,L-phenylalanine-N-methyl-O-methyl-carboxamide

To a stirred solution of compound of Example 68 (1.00 g, 1.92 mmol), O,N-dimethyl hydroxylamine hydrochloride (375 mg, 3.85 mmol), hydroxybenzotriazole hydrate (294 mg, 1.92 mmol) and 4-methylmorpholine (1.06 mL, 9.62 mmol) in tetrahydrofuran (4 mL), cooled in an ice bath, was added EDC (406 mg, 2.12 mmol). The ice bath was removed, and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was diluted with ethyl acetate (75 mL), washed with water, 10% citric acid, water, saturated sodium bicarbonate, and brine. The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum. 750 mg (69% yield) of the title compound was isolated. ^1H NMR (CDCl_3): δ 1.33 (s, 9H), 2.14 (s, 3H), 2.66 (s, 3H), 2.75 (s, 3H), 2.80-2.88 (m, 1H), 3.06-3.20 (m, 4H), 3.70 (s, 3H), 3.84 (s, 3H), 4.98-5.06 (m, 1H), 5.21 (d, $J=8.7$ Hz, 1H), 6.48 (bs, 1H), 6.58 (s, 1H), 7.30-7.34 (m, 2H), 7.60-7.68 (m, 2H), 8.11 (bs, 1H). Analysis calculated for $\text{C}_{27}\text{H}_{38}\text{N}_4\text{O}_7\text{S}\cdot 0.5\text{H}_2\text{O}$: C, 56.73; H, 6.88; N, 9.80. Found: C, 56.97; H, 6.66; N, 9.43.

Example 70Preparation of N- α -Boc-N-omega-4-methoxy-2,3,6-trimethylbenzenesulfonyl-D,L-3-amidinophenylalaninal

- 5 To a stirred solution of LiAlH_4 (2.00 mL of a 1.0M solution in tetrahydrofuran, 1.24 mmol) in tetrahydrofuran (8 mL), cooled in a dry ice/acetone bath, the compound of Example 69 (0.75 g, 1.9 mmol in tetrahydrofuran (5 mL)) was added dropwise. The cooling bath was removed and the
- 10 reaction mixture was allowed to warm to 5°C. The reaction mixture was re-cooled in the dry ice acetone bath and quenched with 3.0 mL of a 1:2.7 wt./wt. solution of potassium bisulfate in water. The reaction mixture was allowed to warm to room temperature, stirred for 3 hours, filtered and
- 15 concentrated under vacuum. The residue was dissolved in ethyl acetate (20 mL) and washed with 10% citric acid (2 mL), water (2 mL), saturated sodium bicarbonate (2 mL) and brine (2 mL). The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed under vacuum to yield 580
- 20 mg (86%) of the title compound. ^1H NMR (CDCl_3): δ 1.31 (s, 9H), 2.07 (s, 3H), 2.57 (s, 3H), 2.67 (s, 3H), 2.90-3.17 (2H, m), 3.77 (s, 3H), 4.33-4.40 (1H, m), 5.02-5.08 (1H, m), 6.48 (1H, s), 7.23-7.31 (2H, m), 7.50-7.62 (2H, m), 7.94, (1H, bs), 8.05 (1H, bs), 9.55 (1H, s). Analysis calculated for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_6\text{S} \cdot 0.5\text{H}_2\text{O}$: C, 58.58; H, 6.69; N, 8.20. Found: C, 58.57; H, 6.72; N, 7.98.
- 25

Example 71Preparation of N- α -Boc-N- ω -4-methoxy-2,3,6-trimethylbenzenesulfonyl-D,L-3-amidinophenylalaninal-semicarbazonyl-4-N-diphenylmethane

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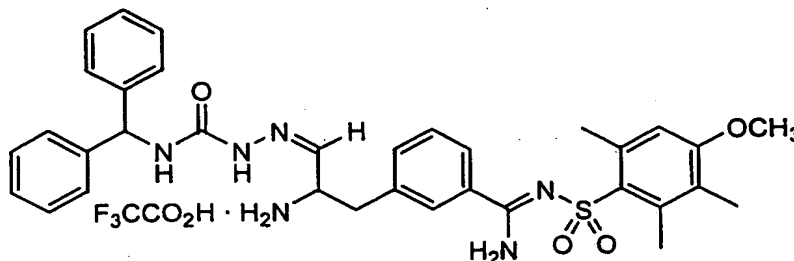
The compound of Example 70 (0.58 g, 1.9 mmol), the compound of Example 65 (410 mg, 1.15 mmol) and sodium acetate trihydrate (188 mg, 1.38 mmol) were refluxed in 75% aqueous ethanol (10 mL) for 1 hour. After the reaction mixture was cooled to room temperature, it was diluted with ethyl acetate (50 mL), washed with 1.0N HCl (5 mL), water (5 mL), saturated sodium bicarbonate (5 mL) and brine (2x5 mL), and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum to yield 750 mg (89% yield) of the title compound as an off-white foam. Analysis calculated for $C_{39}H_{46}N_6O_6S \cdot 1.0 H_2O$: %C, 62.88; %H, 6.49; %N, 11.28. Found: %C, 63.14; %H, 6.35 %N, 11.10. Calculated molecular weight was 726.90.

10

15

Example 72

Preparation of N-omega-4-methoxy-2,3,6-trimethylbenzene sulfonyl-D,L-3-amidinophenylalaninal-semicarbazonyl-4-N-diphenylmethane, trifluoroacetate salt



5

The compound of Example 71 (750 mg, 1.9 mmol) was treated with 50% trifluoroacetic acid/dichloromethane (3 mL) for 30 minutes at room temperature. The reaction mixture was added dropwise to ether (50 mL). The solution was allowed to stand at 4°C for 18 hours. The product was filtered, and dried under vacuum to yield 600 mg (79% yield) of the title compound as an off-white solid. Analysis calculated for $C_{34}H_{38}N_6O_4S \cdot 1.3CF_3CO_2H$: %C, 56.72; %H, 5.11; %N, 10.84. Found: %C, 56.34; %H, 5.47; %N, 11.49. The salt had a calculated molecular weight of 740.8.

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Example 73Preparation of Compound 10-6

Compound 10-6 of Figure 10 is prepared according to the method of Examples 60 to 64 with the substitution of t-butoxycarbonyl-4-(4-pyridyl) alanine in Example 60.

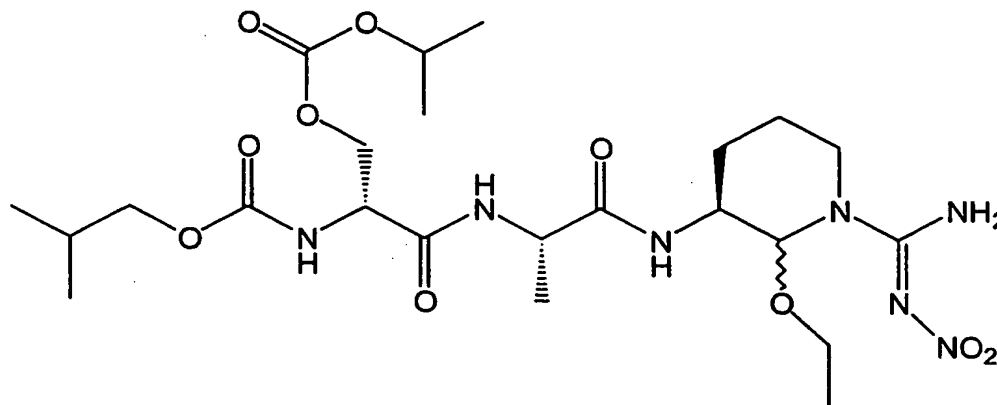
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Example 74Preparation of Compound 11-7

Compound 11-7 of Figure 11 is prepared according to the reaction scheme shown in Figure 11.

Example 75Preparation of Compound 12-5

Compound 12-5 of Figure 12 is prepared according to the reaction scheme shown in Figure 12.

5 Example 76Preparation of i-butoxycarbonyl-D-(isopropoxyloxycarbonyl)Ser-L-Ala-N⁹-nitroargininal-ethyl cyclol

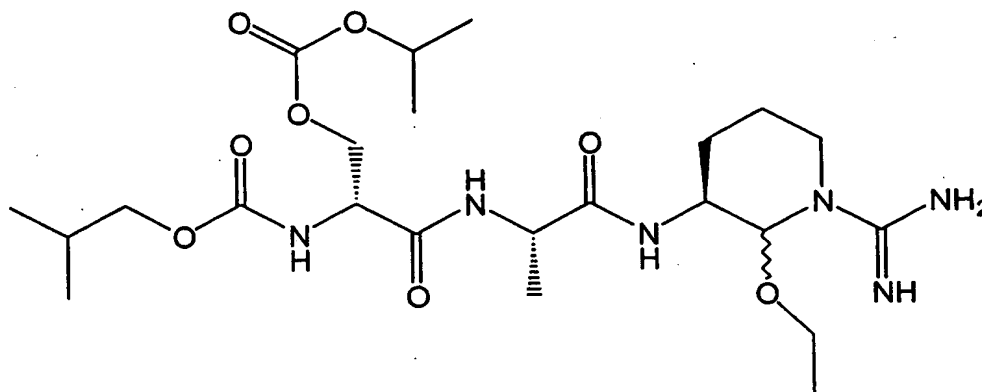
To a solution of the compound of Example 58 (21.0 g, 42.9 mmol) in pyridine (100 mL) cooled in an ice bath, was added isopropyl chloroformate (21 mL of a 1M solution in toluene, 20 mmol, 5 equiv). After 1 hour, the reaction was determined to be complete by analytical HPLC. The reaction mixture was diluted with toluene (300 mL); the solvent was removed under reduced pressure. The residue was dissolved in acetonitrile (400 mL) and the solvent was removed under reduced pressure. The residue was dissolved in acetonitrile (30 mL) and ethyl acetate (30 mL). Ether was added, and the precipitate was isolated. The solid was washed with ether, then dried in vacuo. The solid was washed with ether and ethyl acetate (2 X 200 mL of a 1:1 mixture), then dried in vacuo to give 22.4 g of the title compound in 91% yield as an off-white slightly yellow solid. HPLC: t_r =18.1 minutes in a 5% to 75% acetonitrile gradient in 0.1% aqueous TFA buffer on

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a 4.6 x 250 mm, 5 μ m particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate.

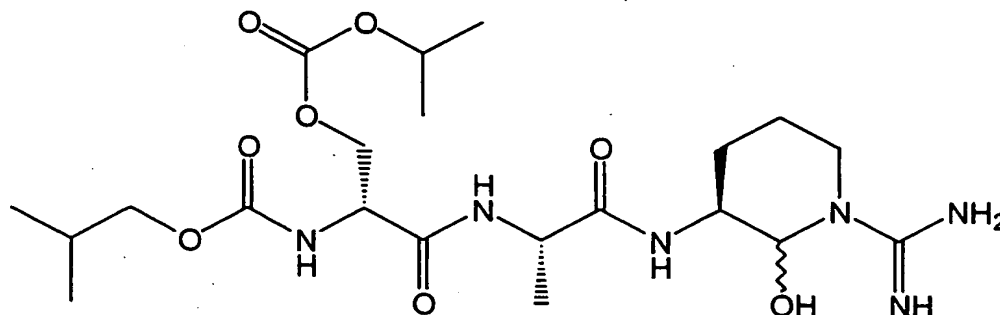
Example 77

Preparation of i-butoxycarbonyl-D-(isopropyloxycarbonyl)Ser-
5 L-Ala-Argininal-ethyl cyclol



A solution of the compound of Example 76 (11.0 g, 19 mmol) in ethanol:water:acetic acid (450 mL of a 4:1:1 mixture) was flushed with nitrogen before 10% palladium on carbon (2.0 g) was added. This mixture was hydrogenated at 35 psi for 6 hours. Celite was added, the reaction mixture was filtered to remove palladium, and solvent was removed in vacuo to give the title compound as a yellowish gummy solid, which was used directly in the procedure described in

15 Example 78. HPLC: t_r =13.1 minutes in a 5% to 75% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 μ m particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate.

Example 78Preparation of i-butoxycarbonyl-D-(isopropoxyloxycarbonyl)Ser-L-Ala-Argininal

5 A solution of the compound of Example 77 (19 mmol) in acetonitrile (20 mL) was treated with 6M HCl (60 mL) for 40 minutes. The reaction mixture was cooled to 0°C, then quenched with sodium acetate (240 mL of a 2.5M solution). The resulting solution was filtered, then purified by
10 preparative HPLC in two lots. The fractions containing product eluted in a 5-40% aqueous acetonitrile containing 0.1% TFA and were pooled and lyophilized yielding 4.1 g (42%) of the title compound as a white powder. Three peaks are observed by analytical HPLC. HPLC: t_r =17.2, 18.2, 18.6
15 minutes in a 5% to 50% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 μ M particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate.

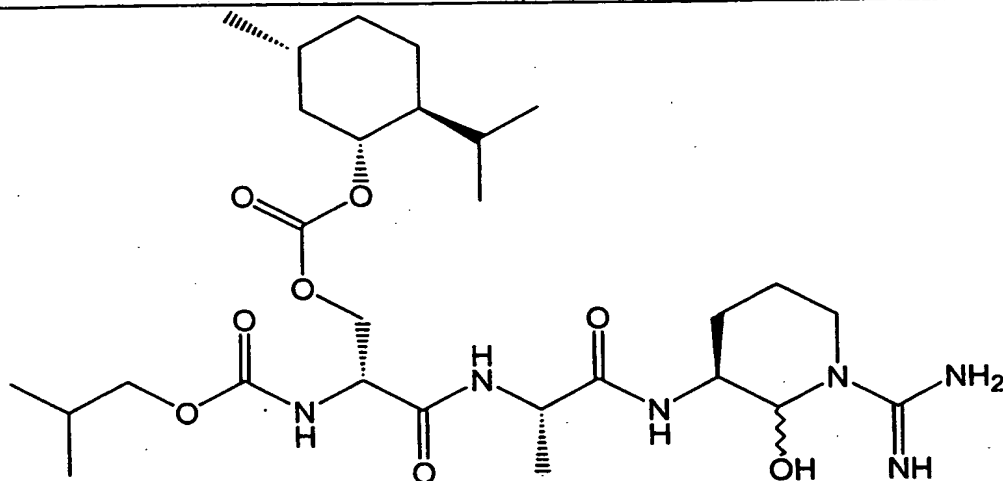
Example 79

20 Following the protocols of Example 76 to 78, with the noted substitution for isopropyl chloroformate, the following compounds were prepared:

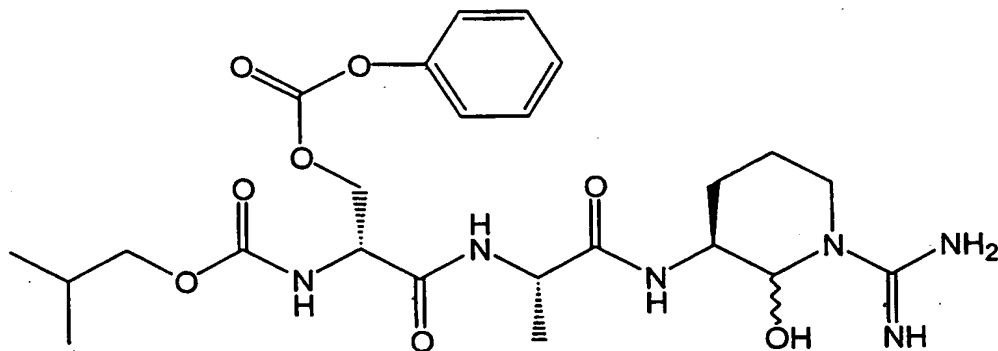
compound name

Substitution in Example 76

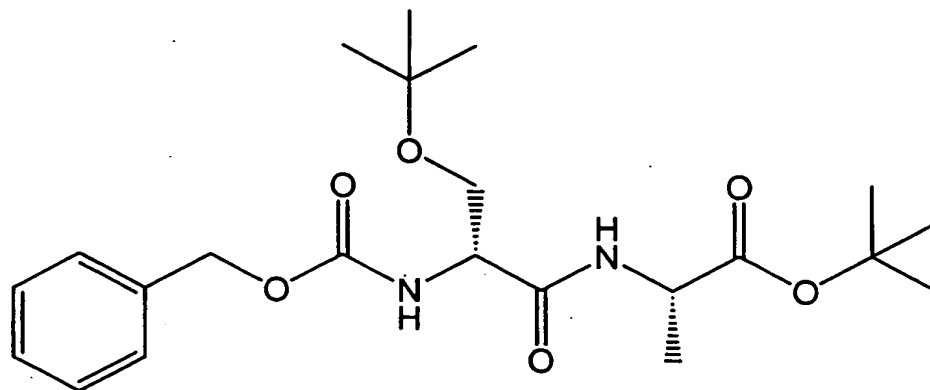
i-butoxycarbonyl-D- (+)-menthyloxycarbonyl) Ser- L-Ala-argininal (Example 79A)	(+)-menthyl chloroformate
i-butoxycarbonyl-D- (phenoxycarbonyl) Ser- L-Ala-Argininal (Example 79B)	phenyl chloroformate



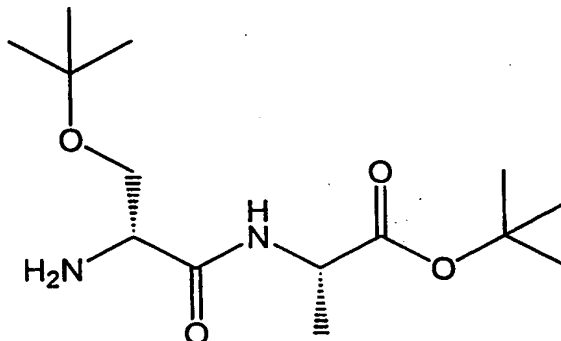
i-butoxycarbonyl-D-((+)-menthyloxycarbonyl) Ser-L-Ala-argininal (Example 79A).



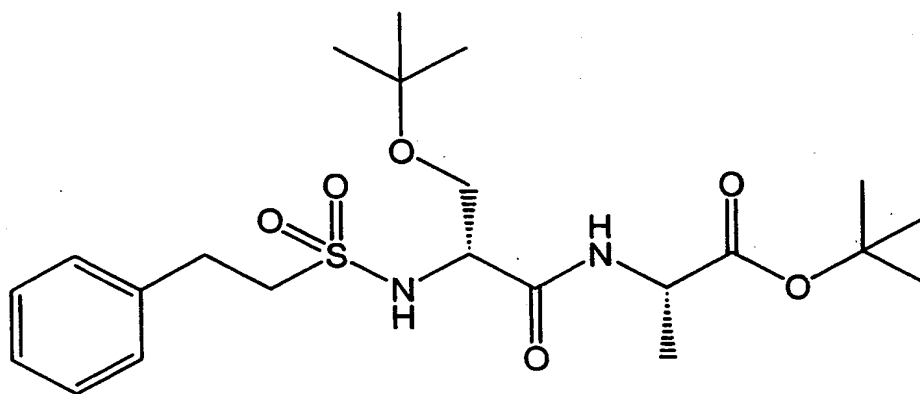
5 i-butoxycarbonyl-D-(phenoxycarbonyl) Ser-L-Ala-argininal (Example 79B).

Example 80Preparation of N- α -benzyloxycarbonyl-D-Ser(O-t-butyl)-L-Ala
t-butyl ester

5 To a solution of N- α -Cbz-D-serine t-butyl ether (5.02 g, 17 mmol) in acetonitrile (100 mL) was added EDC (4.90 g, 25.5 mmol, 1.5 equiv) and 1-hydroxybenzotriazole (2.60 g, 17 mmol). After stirring for 45 minutes, alanine t-butyl ester, HCl salt (3.55 g, 19.6 mmol, 1.15 equiv) and
10 4-methylmorpholine (7.5 mL, 68 mmol, 4 equiv) were added. The reaction mixture was stirred for 1.5 hours. TLC indicated the reaction was complete. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), then washed successively
15 with 1N HCl, saturated sodium bicarbonate, water and brine (25 mL each). The solvent was removed in vacuo to give an oil which crystallized on standing. The title compound (6.95 g) was obtained as a pale yellow solid in 97% yield. R_f = 0.63 (5% isopropanol in dichloromethane).

Example 81Preparation of D-Ser(O-t-butyl)-L-Ala t-butyl ester

A solution of the compound of Example 80 (1.14 g,
5 2.69 mmol) in methanol was hydrogenated over palladium
hydroxide (110 mg) at balloon pressure for 1.5 hours. The
reaction mixture was filtered through celite, and the solvent
was removed in vacuo to afford the title compound in
quantitative yield as a yellow oil. $R_f = 0.44$ (5% methanol
10 in dichloromethane).

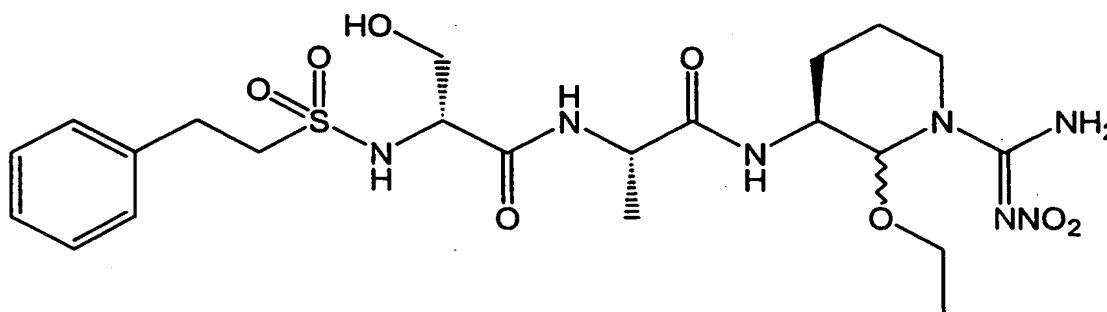
Example 82Preparation of phenethylsulfonyl-D-Ser(O-t-butyl)-L-Ala t-butyl ester

15 To a stirred solution of the compound of Example 81
(4.2 g, 14.6 mmol) and phenethylsulfonyl chloride (3.58 g,
17.5 mmol, 1.2 equiv) in acetonitrile (100 mL) cooled in an
ice bath, was added 2,4,6-collidine (4.8 mL, 36.5 mmol,

2.5 equiv). The reaction mixture was allowed to warm to room temperature, then stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (80 mL); the resulting solution was washed successively with 1.0N HCl, saturated sodium bicarbonate, water and brine, then dried over sodium sulfate. The solvent was removed in vacuo. The residue was chromatographed through silica gel eluting with 0-60% ethyl acetate/hexanes. The title compound was isolated in 89% yield as a yellow oil. $R_f = 0.84$ (5% methanol in dichloromethane).

Example 83

Preparation of phenethylsulfonyl-D-Ser-L-Ala-N^g-nitroargininal-ethyl cyclol



15

The compound of Example 82 (4.16 g, 9.11 mmol) was treated with 50% TFA/dichloromethane (40 mL). After 3 hours, the reaction mixture was diluted with toluene (45 mL) and the solvent was removed under reduced pressure. The residue was dissolved in acetonitrile (40 mL); the solvent was removed in vacuo to afford phenethylsulfonyl-D-serine-L-alanine (4.36 g). $R_f = 0.52$ (10% isopropanol in dichloromethane).

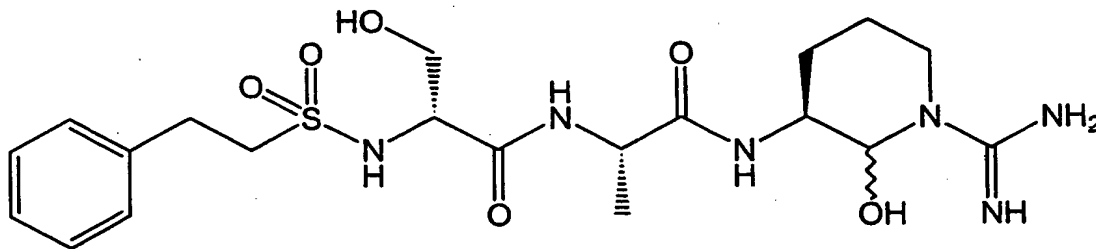
The crude phenethylsulfonyl-D-serine-L-alanine (9.11 mmol, theoretical) was dissolved in acetonitrile

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(90 mL). The stirred solution was cooled in an ice bath while under a nitrogen atmosphere. EDC (3.63 g, 18.9 mmol) and 1-hydroxybenzotriazole (1.93 g, 12.6 mmol) were added; the reaction mixture was allowed to stir for 5 minutes. N⁹-
5 nitroargininal ethyl cyclol, HCl salt (4.04 g, 15.1 mmol) was added. After stirring for 10 minutes, the cooling bath was removed, and 4-methylmorpholine (5.54 mL, 50 mmol) was added. The reaction mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure and then
10 diluted with ethyl acetate. The solution was washed successively with 1.0N HCl, saturated sodium bicarbonate, water and brine, then dried over sodium sulfate, and concentrated under reduced pressure. The combined aqueous layers were back-extracted with ethyl acetate (25 mL). The
15 organic layer was washed successively with 1.0N HCl, saturated sodium bicarbonate, water and brine, then dried over sodium sulfate, and concentrated under reduced pressure. The combined crude product from the extraction and back-extraction were chromatographed through silica gel using 1-6%
20 ethanol in dichloromethane. The title compound was isolated (3.25 g, 64% yield) as an orangish solid. R_f = 0.83 (10% isopropanol in dichloromethane).

Example 84

Preparation of phenethylsulfonyl-D-Ser-L-Ala-argininal



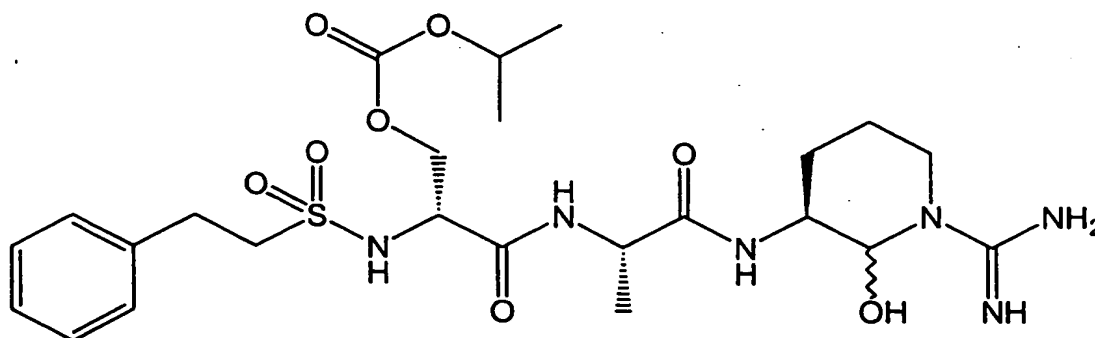
25 Following the two step protocol of hydrogenation and hydrolysis described in Examples 58 to 59, the title compound

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was prepared as a white powder. HPLC: t_r = 15.6 and 17.8 minutes in a 10% to 30% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 μ m particle, 100 angstrom pore, C18 column at a 1 mL/minute flow rate. Mass spec (M+H) = 485.

Example 85

Preparation of phenethylsulfonyl-D-(isopropoxycarbonyl)Ser-L-Ala-argininal



Following the three step protocol of formation of the carbonate, hydrogenation and hydrolysis described in Examples 76 to 78, the title compound was prepared as a white powder. HPLC: t_r = 12.6, 13.1 and 13.2 minutes in a 5% to 90% acetonitrile gradient in 0.1% aqueous TFA buffer on a 4.6 x 250 mm, 5 μ m particle, 100 angstrom pore, C18 column at a 1mL/minute flow rate. Mass spec (M+H) = 571.

By following the teachings of the Detailed Description of the Invention and the Examples and using the appropriate starting materials and reagents, the following compounds were made:

N-alpha-(2-phenylethyl)sulfonyl-D-seryl-azetidine-2-carbonyl-argininal;

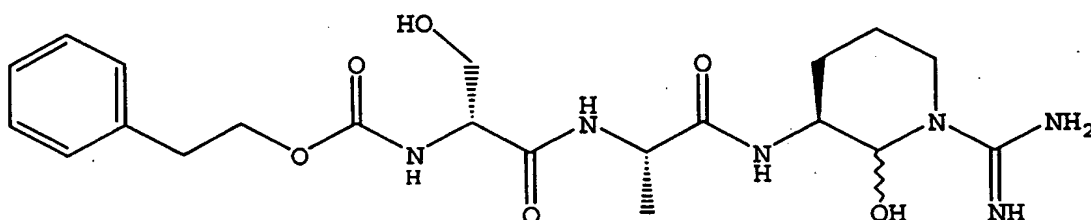
benzyloxycarbonyl-D-seryl-4-hydroxypropyl-argininal; and

benzyloxycarbonyl-D-seryl-3-trans-hydroxypropyl-

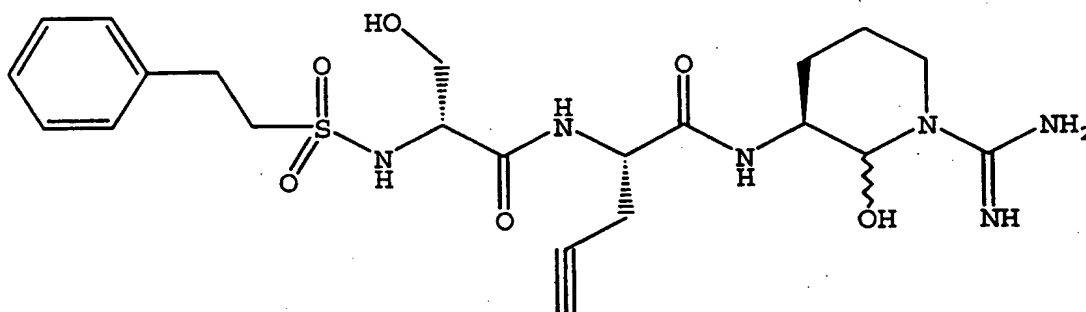
argininal.

By following the teachings of the Detailed Description of the Invention and the Examples and using the appropriate starting materials and reagents, the following compounds are made:

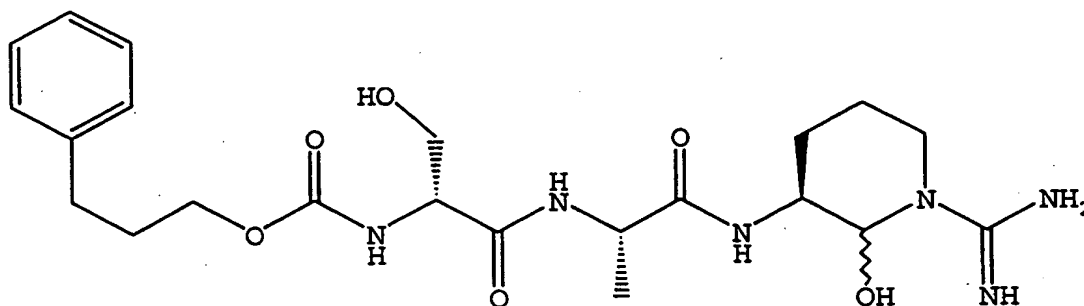
- 5 N-alpha-(2-phenylethyl)sulfonyl-D-seryl-3,4-dihydroprolyl-argininal;



phenethyloxycarbonyl-D-Ser-L-Ala-argininal;

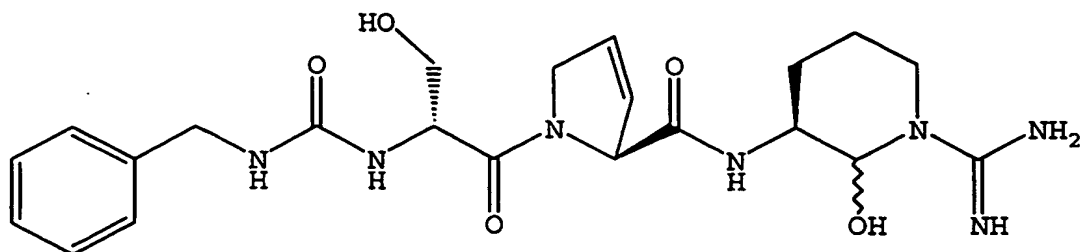


phenethylsulfonyl-D-Ser-L-propargylGly-argininal;

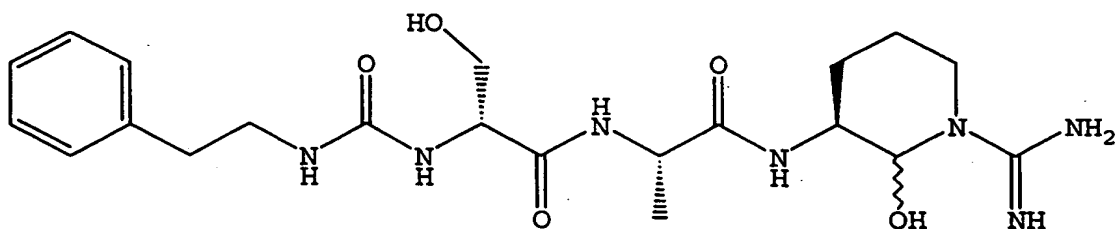


3-phenpropyloxycarbonyl-D-Ser-L-Ala-argininal;

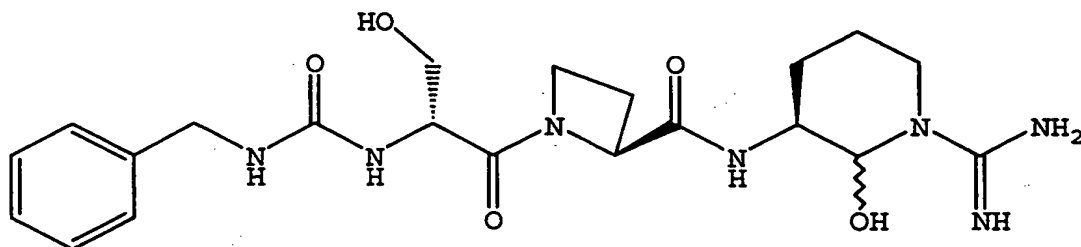
144



benzylaminocarbonyl-D-Ser-L-dehydroprolyl-argininal;

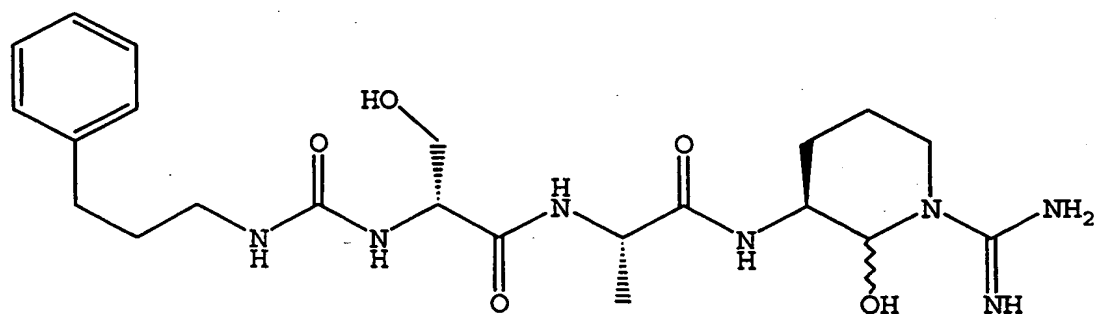


phenethylaminocarbonyl-D-Ser-L-Ala-argininal;



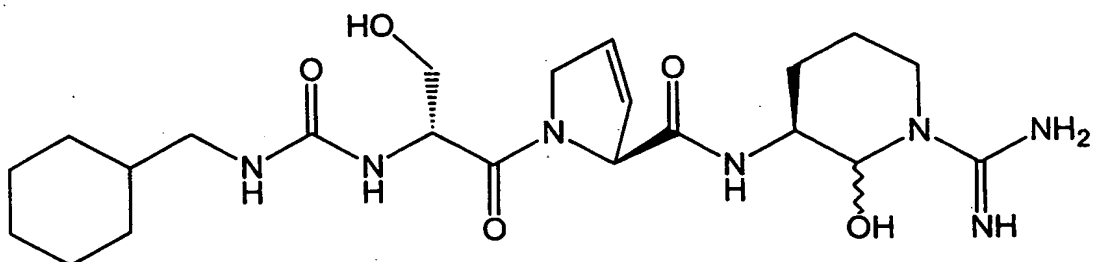
benzylaminocarbonyl-D-Ser-L-azetidine-2-carboxy-argininal;

5

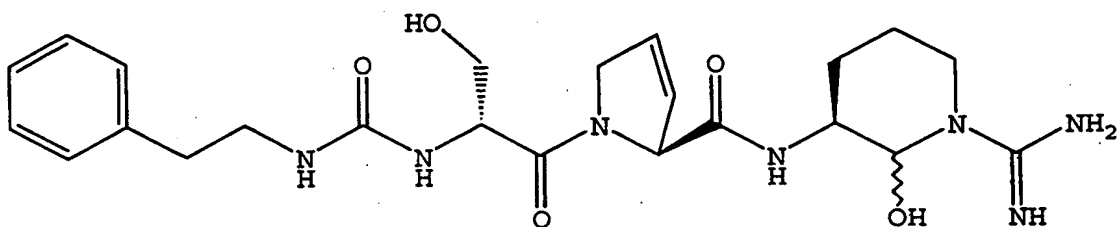


3-phenpropylaminocarbonyl-D-Ser-L-Ala-argininal;

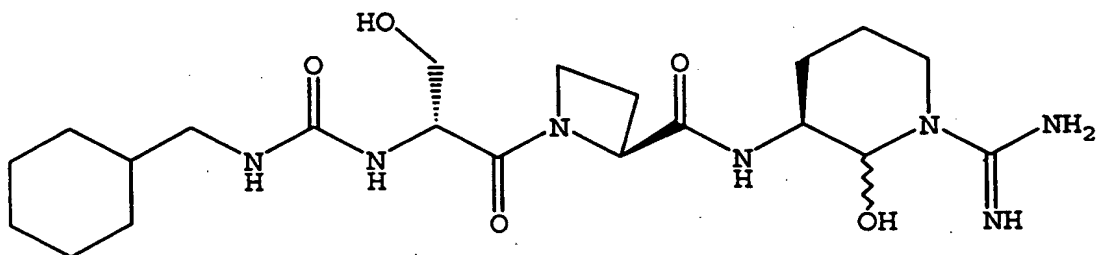
145



cyclohexylmethylaminocarbonyl-D-Ser-L-dehydroprolyl-argininal

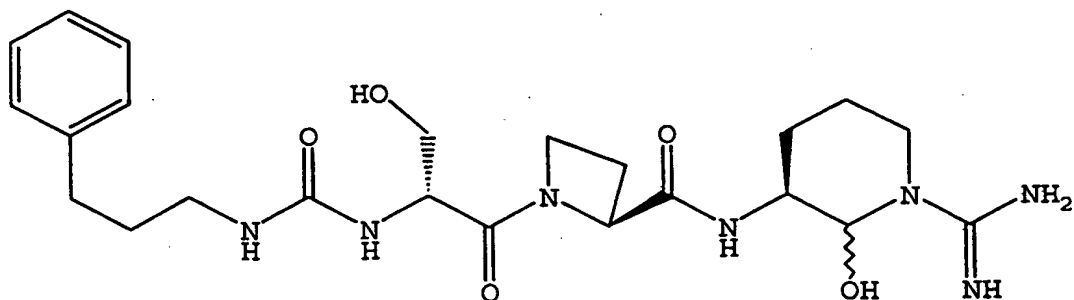


3-phenethylaminocarbonyl-D-Ser-L-dehydroprolyl-argininal;



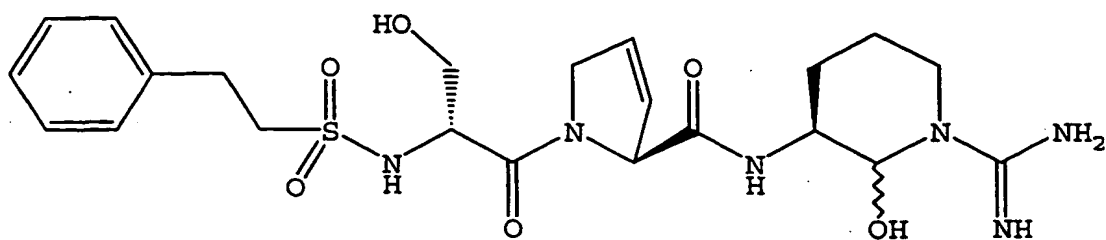
cyclohexylmethylaminocarbonyl-D-Ser-L-azetidine-2-carboxy-argininal

5

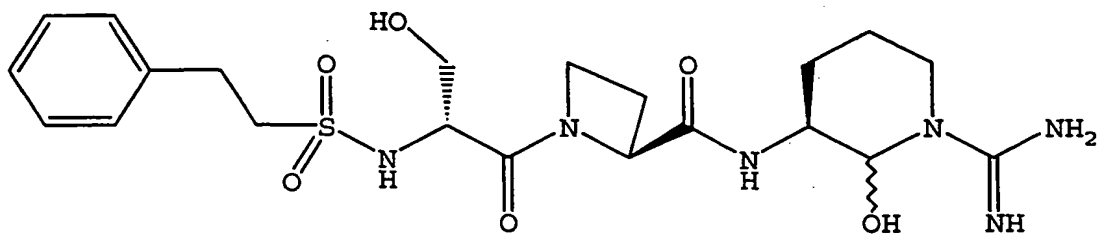


3-phenpropylaminocarbonyl-D-Ser-L-azetidine-2-carboxy-argininal;

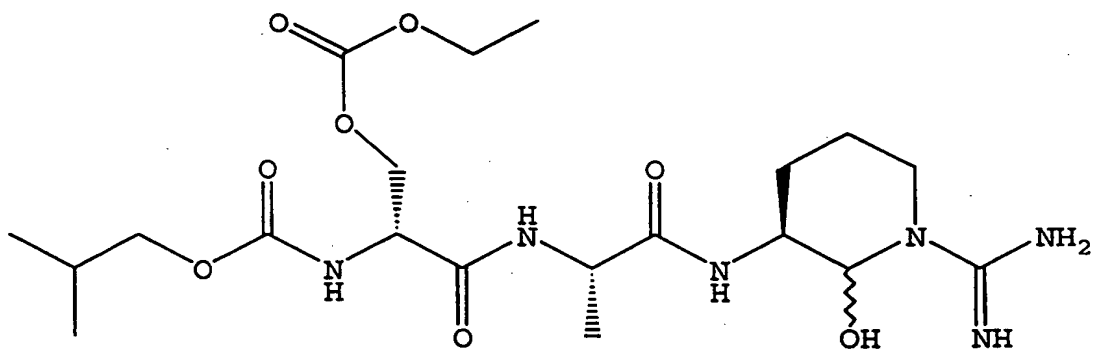
146



phenethylsulfonyl-D-Ser-L-dehydroprolyl-argininal;

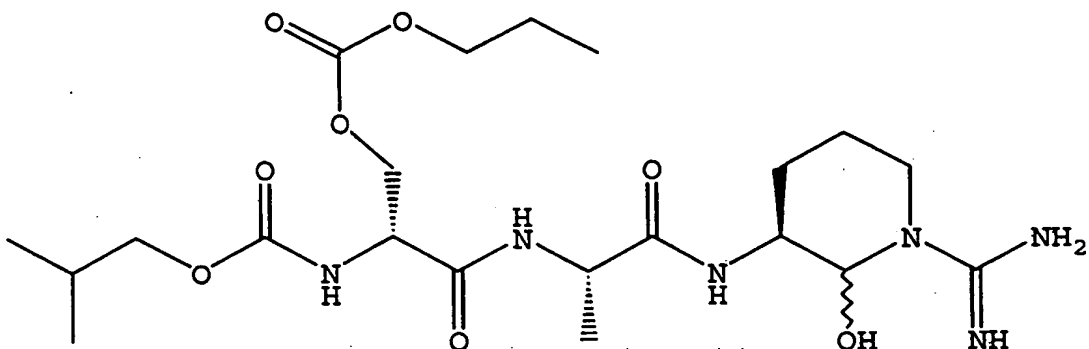


phenethylsulfonyl-D-Ser-L-azetidine-2-carboxy-argininal;

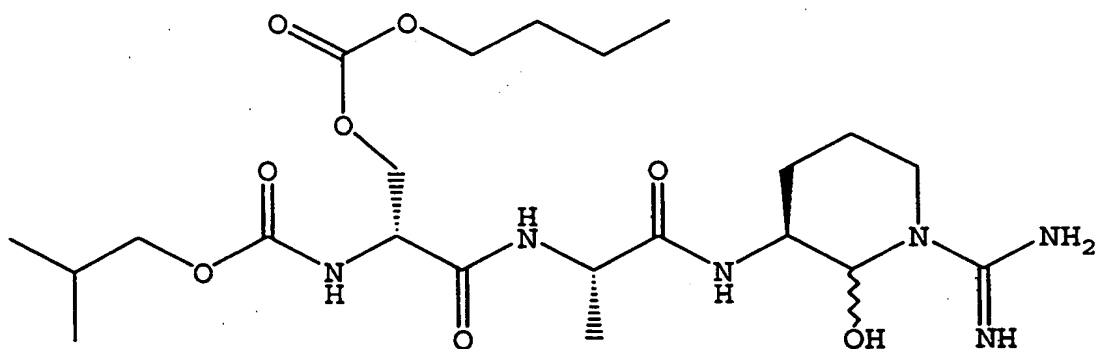


isobutoxycarbonyl-D-(ethoxycarbonyl)-Ser-L-Ala-argininal;

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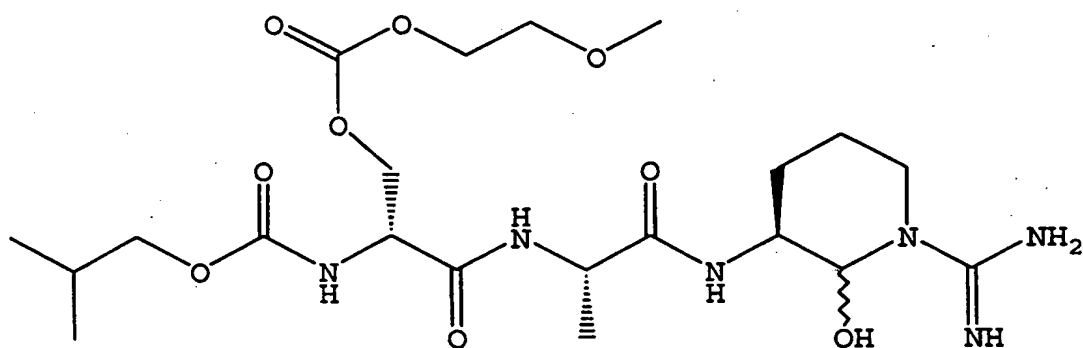


isobutoxycarbonyl-D-(n-propyloxycarbonyl)-Ser-L-Ala-argininal;



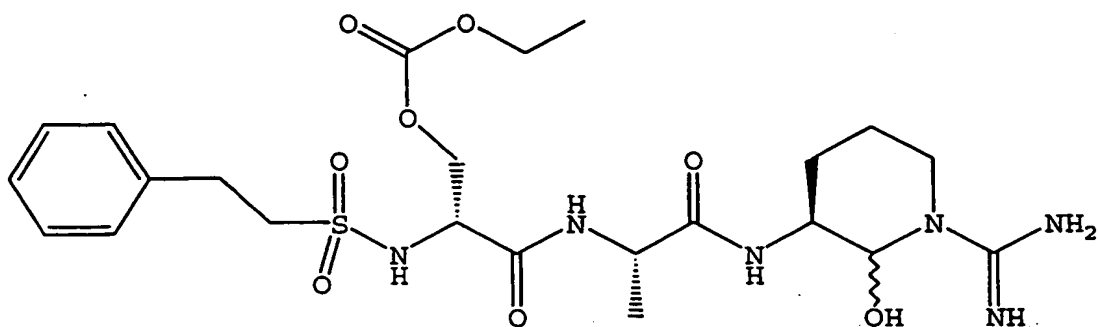
isobutoxycarbonyl-D-(n-butyloxycarbonyl)-Ser-L-Ala-argininal;

5

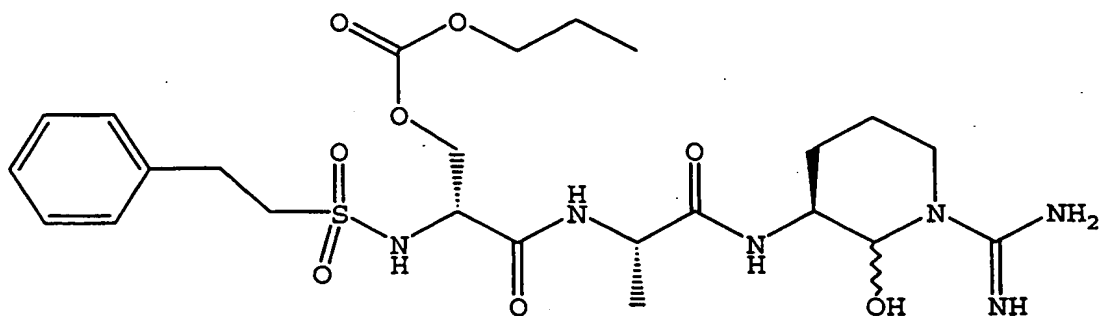


isobutoxycarbonyl-D-(2-methoxyethoxycarbonyl)-Ser-L-Ala-argininal;

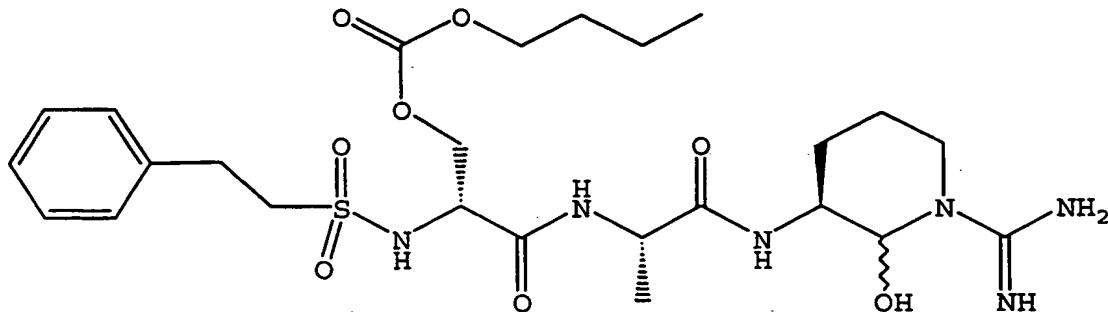
148



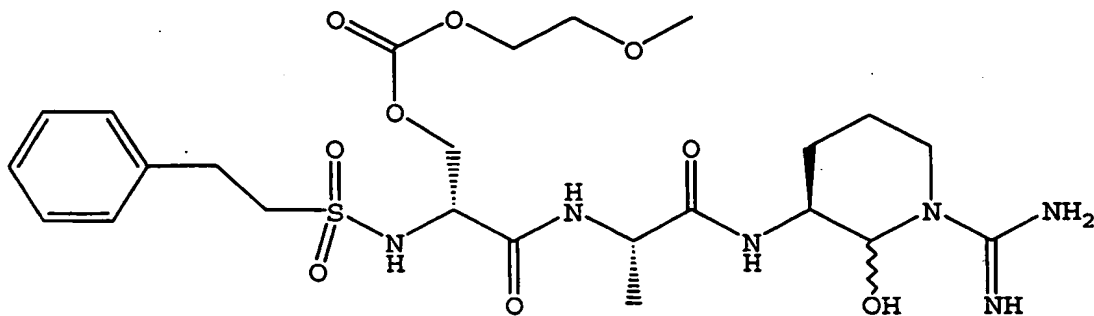
phenethylsulfonyl-D-(ethoxycarbonyl)-Ser-L-Ala-argininal;



phenethylsulfonyl-D-(n-propyloxycarbonyl)-Ser-L-Ala-argininal;



phenethylsulfonyl-D-(n-butyloxycarbonyl)-Ser-L-Ala-argininal; and



phenethylsulfonyl-D-(2-methoxyethoxycarbonyl)-Ser-L-Ala-argininal.

Example AIn vitro enzyme Assays for specificity determination

The ability of compounds of the present invention to act as selective inhibitors of urokinase catalytic activity was assessed by determining the concentration of test compound which inhibited the activity of this enzyme by 50%, (IC_{50}), and comparing this value to that determined for all or some of the following related serine proteases: recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, factor Xa, thrombin and trypsin.

The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

The assay for IC_{50} determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration (covering a broad concentration range) diluted in HBSA (or HBSA alone for V_0 (uninhibited velocity) measurement), and 50 microliters of the enzyme diluted in HBSA. Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate at the concentrations specified below were added to the wells, yielding a final total volume of 200 microliters (about 4 times K_m). The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

Urokinase Assay

Urokinase catalytic activity was determined using the chromogenic substrate 150 mM S-2444 (L-Pyroglutamyl-glycyl-L-arginine-p-nitroaniline hydrochloride), obtained from
5 DiaPharma Group, Inc. Urokinase (Abbokinase), manufactured by Abbott Laboratories, was obtained from Priority Pharmaceuticals and diluted to 750 pM in the HBSA assay buffer prior to use. The assay buffer was HBS (10mM HEPES, 150mM sodium chloride pH 7.4) with 0.1% BSA.

10 Thrombin (fIIa) Assay

Enzyme activity was determined using the chromogenic substrate, Pefachrome t-PA (CH_3SO_2 -D-hexahydrotyrosine-glycyl-L-Arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was reconstituted in deionized water
15 prior to use. Purified human α -thrombin was obtained from Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

IC_{50} determinations were conducted where HBSA (50 μL),
20 α -thrombin (50 μl) (the final enzyme concentration is 0.5 nM) and inhibitor (50 μl) (covering a broad concentration range), were combined in appropriate wells and incubated for 30 minutes at room temperature prior to the addition of substrate Pefachrome-t-PA (50 μl) (the final substrate
25 concentration is 250 μM , about 5 times K_m). The initial velocity of Pefachrome t-PA hydrolysis was measured by the change in absorbance at 405nm using a Thermo Max[®] Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of
30 added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

Factor Xa

Factor Xa catalytic activity was determined using the chromogenic substrate S-2765 (N-benzyloxycarbonyl-D-arginine-L-glycine-L-arginine-p-nitroaniline), obtained from
5 DiaPharma Group (Franklin, OH). All substrates were reconstituted in deionized water prior to use. The final concentration of S-2765 was 250 μ M (about 5-times K_m). Purified human Factor X was obtained from Enzyme Research Laboratories, Inc. (South Bend, IN) and Factor Xa (FXa) was
10 activated and prepared from it as described [Bock, P.E., Craig, P.A., Olson, S.T., and Singh, P., Arch. Biochem. Biophys. 273:375-388 (1989)]. The enzyme was diluted into HBSA prior to assay in which the final concentration was 0.25 nM.

15 Recombinant tissue plasminogen activator (rt-PA) Assay

rt-PA catalytic activity was determined using the substrate, Pefachrome t-PA (CH_3SO_2 -D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed
20 by dilution in HBSA prior to the assay in which the final concentration was 500 micromolar (about 3-times K_m). Human rt-PA (Activase®) was obtained from Genentech Inc. The enzyme was reconstituted in deionized water and diluted into HBSA prior to the assay in which the final concentration was
25 1.0 nM.

Plasmin Assay

Plasmin catalytic activity was determined using the chromogenic substrate, S-2366 [L-pyroglutamyl-L-prolyl-L-arginine-p-nitroaniline hydrochloride], which was obtained
30 from DiaPharma Group. The substrate was made up in deionized

water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 2.5-times K_m). Purified human plasmin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA
5 prior to assay in which the final concentration was 1.0 nM.

Activated Protein C (aPC) Assay

aPC catalytic activity was determined using the chromogenic substrate, Pefachrome PC (delta-carbobenzyloxy-D-lysine-L-prolyl-L-arginine-p-nitroaniline dihydrochloride),
10 obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 400 micromolar (about 3 times K_m). Purified human aPC was obtained from Hematologic Technologies, Inc. The enzyme was diluted into
15 HBSA prior to assay in which the final concentration was 1.0 nM.

Chymotrypsin Assay

Chymotrypsin catalytic activity was determined using the chromogenic substrate, S-2586 (methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide), which was obtained from
20 DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 100 micromolar (about 9-times K_m). Purified (3X-crystallized; CDI) bovine pancreatic
25 α -chymotrypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5 nM.

Trypsin Assay

Trypsin catalytic activity was determined using the chromogenic substrate, S-2222 (benzoyl-L-isoleucine-L-glutamic acid-[gamma-methyl ester]-L-arginine-p-nitroanilide), which was obtained from DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 4-times K_m). Purified (3X-crystallized; TRL3) bovine pancreatic trypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5 nM.

Table I

Table I lists the determined IC_{50} values for certain of the enzymes listed above for compounds of the present invention that demonstrate a high degree of specificity for the inhibition of urokinase compared to other serine proteases.

TABLE I

Compound No.	Example No.	uPA*	tPA*	plasmin*
1	8 35-40 54-61	A	D	C
2	9	A	C	C
3	10	A	D	NT
4	10	A	D	NT
5	10	A	D	NT
6	10	A	D	NT

Compound No.	Example No.	uPA*	tPA*	plasmin*
7	10	A	D	NT
8	10	A	D	NT
9	10	A	D	NT
10	11	A	D	NT
11	12	A	D	C
12	12	A	D	C
13	12	B	D	NT
14	12	A	D	NT
15	12	B	D	NT
16	12	B	D	NT
17	12	B	D	NT
18	12	C	D	NT
19	13	A	D	C
20	13	B	D	NT
21	13	B	NT	NT
22	13	C	D	NT
23	13	C	D	NT
24	14	B	NT	NT
25	14	B	NT	NT
26	15	B	D	NT
27	15	B	D	A
28	16-20	A	D	C
29	21-26	A	D	C
30	27-34	B	D	C
31	46	A	NT	C
32	47-50	A	D	C
35	10	C	D	D
36	10	A	D	C
37	10	B	D	D

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Compound No.	Example No.	uPA*	tPA*	plasmin*
38	10	B	D	D
39	10	C	D	D
40	10	A	D	D
41	16	C	D	C
42	16	B	D	B
43	16	C	D	C
44	51	C	D	C
45	52	B	D	C
46	52	A	D	C
47	51	A	D	C
48	51	A	D	C
49	52	A	D	C
50	52	A	D	B
51	52	A	D	C
52	52	B	D	C
53	53	A	D	C

*

A = less than 100 nm

B = 100-250 nm

C = 250-2500 nm

5 D = greater than 2500 nm

NT = Not tested

Example B

Evaluation of Compound 1 as an Inhibitor of Angiogenesis In Vivo

The chicken CAM (chick embryo chorioallantoic membrane) model, a standard angiogenesis assay, was used to evaluate the ability of Compound 1 to inhibit angiogenesis. This model is an established model for evaluation of activity of a test compound to affect formation of new blood vessels.

A filter disc saturated with a 0.5 µg/ml solution of basic fibroblast growth factor (bFGF) was placed on the CAM of 10 day old chick embryos to induce angiogenesis. Twenty four hours later, 0 to 1 µg of Compound 1, in a total volume of 100 µl of sterile PBS, was injected intravenously into the embryo. Approximately 48 hours later, the embryos were sacrificed and the filter discs and surrounding CAM tissue were excised for analysis. Angiogenesis was quantitated by counting the number of blood vessel branch points within the confined region of the filter [Brooks, P.C., et al, Methods in Molecular Biology 120:257-269 (1999)]. The angiogenic index is defined as the difference in the number of blood vessel branch points between an experimental group and the untreated, control embryos. Each experimental group contained 8 to 10 chicken embryos. A single administration of 1 µg of Compound 1 inhibited angiogenesis by 92% in this experiment.

Table II

<u>Inhibition of Cytokine-Induced Angiogenesis by Compound 1</u>		
<u>Treatment</u>	<u># of Blood Vessel Branch Points</u>	<u>Angiogenic Index</u>
None	13 +/- 2	0
bFGF	39 +/- 8	26
bFGF, 0.001 µg Compound 1	35 +/- 5	22
bFGF, 0.01 µg Compound 1	21 +/- 5	8
bFGF, 1.0 µg Compound 1	15 +/- 1	2

Example C5 Evaluation of Compound 1 to Inhibit the Growth of Human Tumor Cells in a Chick Embryo Model

A chicken embryo model was used to evaluate the activity of Compound 1 to inhibit the growth of human tumor cells in vivo. A single cell suspension of human fibrosarcoma cells (HT 1080), containing 4×10^5 cells in a total volume of 40 µl, was applied to 10 day old chick embryos as described by Brooks, et al ("Brooks, P.C., et al, "Integrin $\alpha_v\beta_3$ Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels", Cell 79:1157-1164 (1994)).

15 Twenty-four hours later 0 to 10 µg of Compound 1 was injected intravenously into the embryos. Following this single administration of compound, control and treated embryos were incubated for a total of 7 days and then sacrificed. Tumors were excised, trimmed free of surrounding CAM tissue, and

20 weighed. Table III lists the wet weights for tumors excised

in this experiment. Each experimental group contained 10 to 12 chicken embryos. A single dose of 10 μ g of Compound 1 reduced tumor weight by approximately 65%.

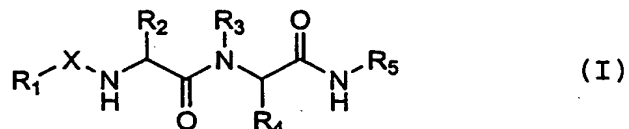
5

Table III

<u>Inhibition of the Growth of HT1080 Cells in Chicken Embryos by Compound 1</u>	
<u>Treatment</u>	<u>Wet weight (mg)</u>
Control	273 +/- 49
Compound 1 (1 μ g)	174 +/- 25
Compound 1 (10 μ g)	97 +/- 15

Claims

1. A compound of the formula:



wherein:

- 5 (a) X is selected from the group consisting of
 -S(O)₂-, -N(R')-S(O)₂-, -(C=O)-, -OC(=O)-, -NH-C(=O)-,
 -P(O)(R')-, and a direct link, wherein R' is independently
 hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6
 10 to about 14 carbon atoms or aralkyl of about 7 to about 16
 carbon atoms, with the proviso that when X is -P(O)(R')-,
 then R' is not hydrogen;
- (b) R₁ is selected from the group consisting of:
- (1) alkyl of 1 to about 12 carbon atoms which
 is optionally substituted with Y₁,
- 15 (2) alkyl of 1 to about 3 carbon atoms
 substituted with cycloalkyl of about 5 to about 8 carbon
 atoms which is optionally mono-, di- or tri-substituted on
 the ring with Y₁, Y₂, and/or Y₃,
- (3) cycloalkyl of 3 to about 15 carbon atoms,
 20 which is optionally mono-, di-, or tri-substituted on the
 ring with Y₁, Y₂, and/or Y₃,
- (4) heterocycloalkyl of 4 to about 10 ring
 atoms with the ring atoms selected from carbon and
 heteroatoms, wherein the heteroatoms are selected from the
 25 group consisting of oxygen, nitrogen, and S(O)_i, wherein i is
 0, 1 or 2, which is optionally mono-, di-, or tri-substituted
 on the ring with Y₁, Y₂, and/or Y₃,
- (5) heterocyclo of 4 to about 10 ring atoms
 with the ring atoms selected from carbon and heteroatoms,

wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including, $-\text{N} \begin{smallmatrix} \circ \\ \text{V} \end{smallmatrix}$ wherein $-\text{N} \begin{smallmatrix} \circ \\ \text{V} \end{smallmatrix}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is
5 -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y₁, Y₂, and/or Y₃,

(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of about 5 to
10 about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y₁, Y₂, and/or Y₃,

15 (8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y₁, Y₂, and/or Y₃,

20 (9) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y₁, Y₂, and/or Y₃,

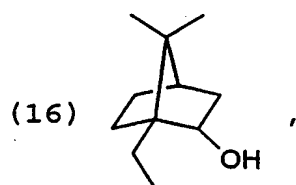
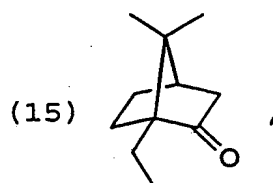
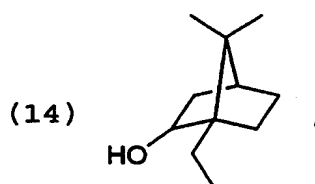
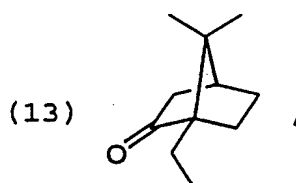
(10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and
25 heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

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(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

5 (12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,

10



15 (17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms;

(18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,

20 (19) perfluoroaryl of about 6 to about 14 carbon atoms,

(20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(21) hydrogen when X is a direct link;
wherein each Y_1 , Y_2 , and Y_3 is independently selected and is

5 (i) selected from the group consisting of halogen, cyano, nitro, tetrazolyl, guanidino, amidino, methylguanidino, $-CF_3$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2H$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$,
10 $-C(O)OZ_1$, $-C(O)NH_2$, $-C(O)NHZ_1$, $-C(O)NZ_1Z_2$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, $-NZ_1Z_2$, $-N$ -morpholino, $-S(CF_2)_qCF_3$, and $-S(O)_m(CF_2)_qCF_3$, wherein m is 0, 1 or 2, q is an integer from 0 to 5, and Z_1 and Z_2 are independently selected from the group consisting
15 of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms, or

(ii) Y_1 and Y_2 are selected together to
20 be $-O[C(Z_3)(Z_4)]_rO-$, wherein r is an integer from 1 to 4 and Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15
25 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms;

(c) R_2 is selected from the group consisting of H, $-CH_3$, $-C_2H_5$, $-(CH_2)_2OH$, $-(CH_2)_2OA_2$, $-CH(R_6)OH$, $-CH(R_6)OA_2$ and $-CH_2NH-X'-R_6$ wherein A_2 is $-C(=O)OR_9$ or $-C(=O)R_9$; X' is
30 selected from the group consisting of $-S(O)_2-$, $-S(O)_2-N(R'')-$, $-(C=O)-$, $-C(=O)-O-$, $-C(=O)-NH-$, $-P(O)(R'')$, and a direct link, wherein R'' is hydrogen, alkyl of 1 to about 4 carbon

atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms with the proviso that when X' is -P(O)(R'')-, then R'' is not hydrogen; R₆ is selected from the group consisting of:

5 (1) alkyl of 1 to about 12 carbon atoms, optionally substituted with Y₁,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on
10 the ring with Y₁, Y₂, and/or Y₃,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y₁, Y₂, and/or Y₃,

(4) heterocycloalkyl of 4 to about 10 ring
15 atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y₁, Y₂, and/or Y₃,

20 (5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and S(O)_i, wherein i is 0, 1, or 2, including $-\text{N} \begin{smallmatrix} \text{---} \end{smallmatrix} \text{V}$, wherein $-\text{N} \begin{smallmatrix} \text{---} \end{smallmatrix} \text{V}$ is a 5 to 7 member
25 heterocycle having 3 to 6 ring carbon atoms, where V is -CH₂-, -O-, -S(=O)-, -S(O)₂- or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y₁, Y₂, and/or Y₃,

(6) alkenyl of 2 to about 6 carbon atoms
30 which is optionally substituted with cycloalkyl of about 5 to

about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(9) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 , and

(13) hydrogen; and

R_9 is selected from the group consisting of:

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(1) alkyl of 1 to about 12 carbon atoms, optionally substituted with Y_1 ,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 5 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 ,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including $-\text{N} \begin{smallmatrix} \text{---} \end{smallmatrix} \text{V}$, wherein $-\text{N} \begin{smallmatrix} \text{---} \end{smallmatrix} \text{V}$ is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}(=\text{O})-$, $-\text{S}(\text{O})_2-$ or $-\text{S}-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1 , Y_2 , and/or Y_3 ,

(6) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from

oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1 , Y_2 , and/or Y_3 ,

(8) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1 , Y_2 , and/or Y_3 ,

(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1 , Y_2 , and/or Y_3 , and

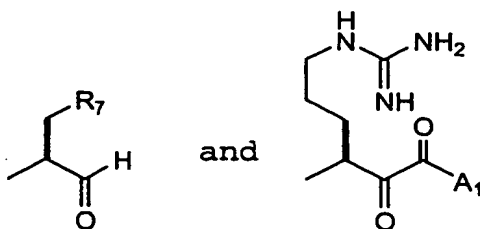
(10) hydrogen, with the proviso that R_9 is not hydrogen when A_2 is $-C(=O)OR_9$;

(d) R_3 is selected from H or methyl, or R_3 and R_4 are selected together as set forth in (f);

(e) R_4 is in the S configuration and is selected from the group consisting of H, $-CH_2-S-CH_3$, $-CH_2OH$, $-CH_2CN$, lower alkyl of 1 to about 3 carbon atoms, $-CH_2C\equiv CH$, $-CH_2CH=CH_2$ and $-CH=CH_2$ or R_3 and R_4 are selected together as set forth in (f);

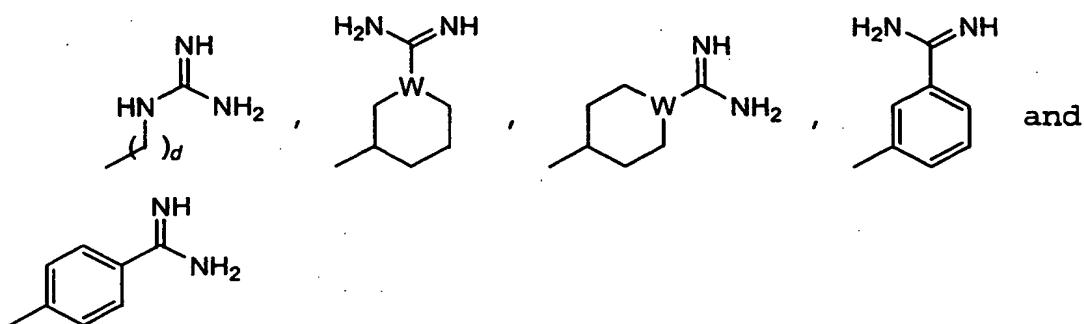
(f) alternatively, R_3 and R_4 are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecholy, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, and 3,4-dehydroprolyl;

(g) R_5 is selected from the group consisting of



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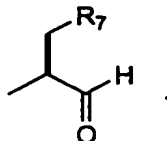
wherein R_7 is selected from



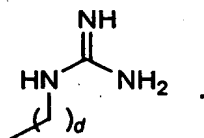
wherein d is an integer from 1 to 3 and W is $-N-$ or $-CH-$; and

- 5 (h) A_1 is $-NHR_8$, wherein R_8 is alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 6 to about 15 carbon atoms, all optionally mono-, di or tri-substituted with Y_1 , Y_2 and/or Y_3 or is hydrogen; and pharmaceutically acceptable salts thereof.

- 10 2. A compound according to claim 1 wherein R_5 is:



3. A compound according to claim 2 wherein R_7 is:



4. A compound according to claim 3 wherein d is 2.

- 15 5. A compound according to claim 4 wherein R_2 is H , $-CH_3$, $-CH_2CH_3$, $-CH_2NH(X')(R_6)$ or $-CH(R_6)OH$.

6. A compound according to claim 5 wherein R_6 is hydrogen, alkyl optionally substituted with Y_1 or aralkyl

optionally mono-, di- or tri-substituted with Y_1 , Y_2 and/or Y_3 .

7. A compound according to claim 6 wherein R_2 is H, $-CH_2OH$ or $-CH(CH_3)OH$.

5 8. A compound according to claim 7 wherein R_2 is $-CH(CH_3)OH$ and has the R configuration.

9. A compound according to claim 7 wherein R_3 is hydrogen.

10 10. A compound according to claim 9 wherein R_4 is methyl or propargyl.

11. A compound according to claim 7 wherein R_3 and R_4 are selected together to give a group at P_2 selected from the group consisting of prolyl, pipecholy, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl and 3,4-dehydroprolyl.

15 12. A compound according to claim 11 wherein R_3 and R_4 are selected together to give a group at P_2 selected from the group consisting of prolyl, 4-cis-hydroxyprolyl, 3,4-dehydroprolyl and azetidine-2-carbonyl.

13. A compound according to claim 5 wherein R_6 is H.

20 14. A compound according to claim 1 wherein X is selected from $-S(O)_2-$, $-OC(=O)-$, $-NH-C(=O)-$ and a direct link.

15. A compound according to claim 14 wherein X is $-S(O)_2-$ or $-OC(=O)-$.

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16. A compound according to claim 15 wherein R_1 is selected from phenyl, benzyl, 2-phenylethyl, isobutyl and 3-phenylpropyl.

17. A compound according to claim 16 wherein R_1 -X- is
5 selected from phenyl-S(O)₂-, benzyl-S(O)₂-,
2-phenylethyl-S(O)₂-, 3-phenylpropyl-S-(O)₂-, benzyl-OC(=O)-
and isobutyl-OC(=O)-.

18. A compound according to claim 1 wherein R_2 is
10 selected from hydrogen, methyl, ethyl, -CH₂NH(X') (R₆) and
-CH(R₆)OH.

19. A compound according to claim 18 wherein R_6 is selected from hydrogen, alkyl optionally substituted with Y_1 and aralkyl optionally mono-, di- or tri-substituted with Y_1 , Y_2 and/or Y_3 .

15 20. A compound according to claim 19 wherein R_2 is selected to give a group at P3 selected from glycyl, D-seryl, (R,R)D-allothreonyl, D-2-aminobutyryl, N-β-methyloxycarbonyl-D-2,3-diaminopropionyl, N-β-(2-phenylethylcarbonyl)-D-2,3-diaminopropionyl, and
20 N-β-benzyloxycarbonyl-D-2,3-diaminopropionyl.

21. A compound according to 20 wherein P3 is D-seryl or (R,R)D-allothreonyl.

22. A compound according to claim 1 wherein R_3 is hydrogen.

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23. A compound according to claim 1 wherein R_4 is methyl or propargyl.

24. A compound according to claim 1 wherein R_3 and R_4 are selected together to give a group at P2 selected from
5 prolyl, pipecholy, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl and 3,4-dehydroprolyl.

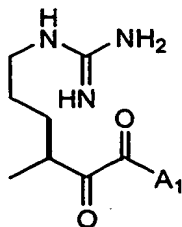
25. A compound according to claim 24 wherein R_3 and R_4 are selected together to give a group at P2 selected from
10 prolyl, 4-cis-hydroxyprolyl, 3,4-dehydroprolyl and azetidine-2-carbonyl.

26. A compound according to claim 1 wherein R_2 is selected to give a group at P3 selected from D-seryl and (R,R)D-allothreonyl, and R_5 is selected to give an arginine aldehyde at P1.

15 27. A compound according to claim 26 wherein R_3 is hydrogen and R_4 is methyl.

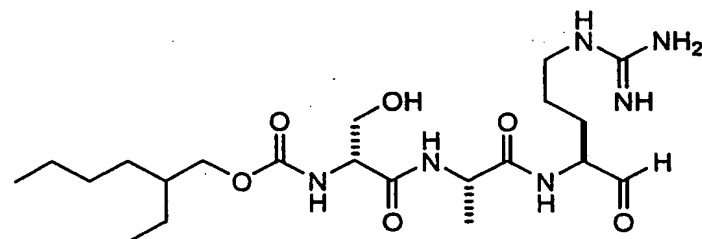
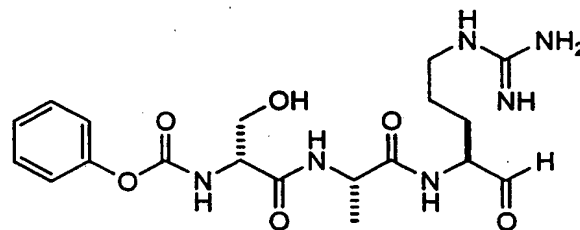
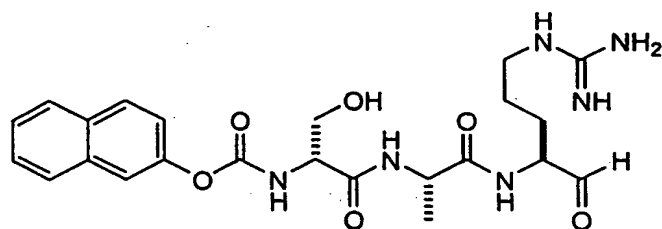
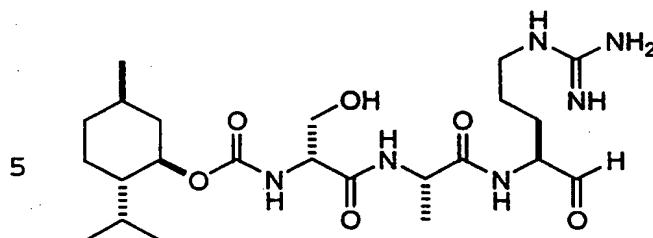
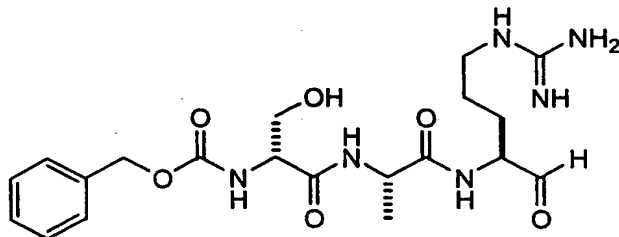
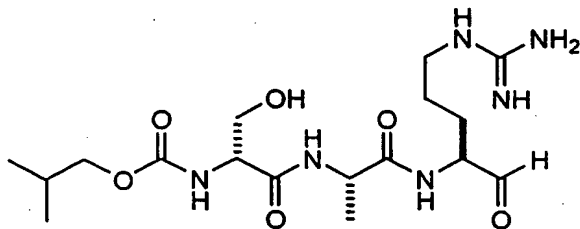
28. A compound according to claim 26 wherein R_3 and R_4 are selected together to give a group at P2 selected from prolyl, azetidine-2-carbonyl and 3,4-dehydroprolyl.

20 29. A compound according to claim 1 wherein R_5 is

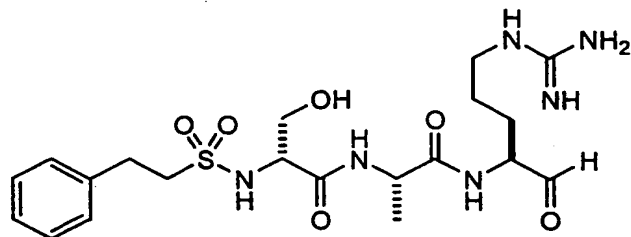
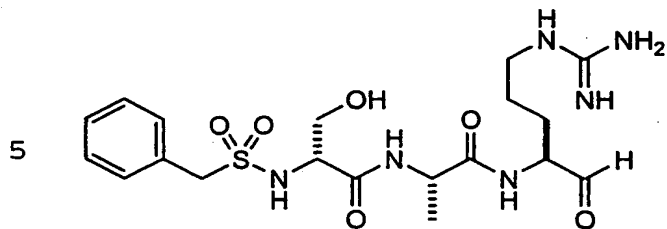
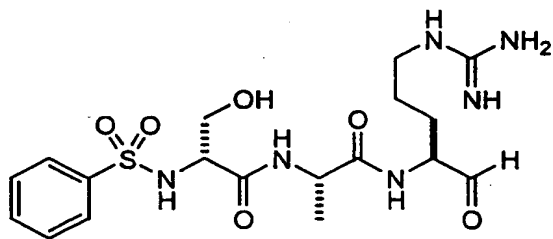
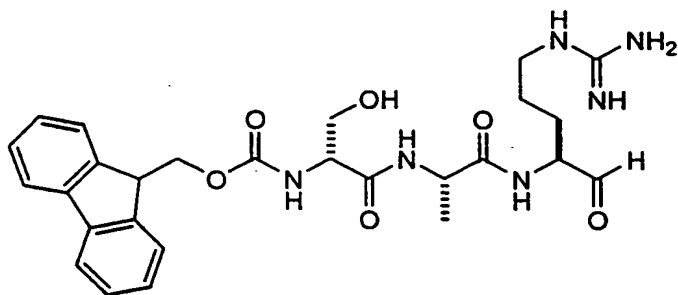
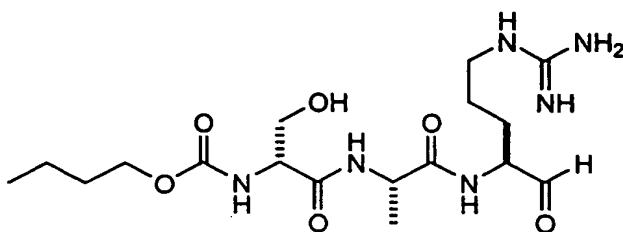
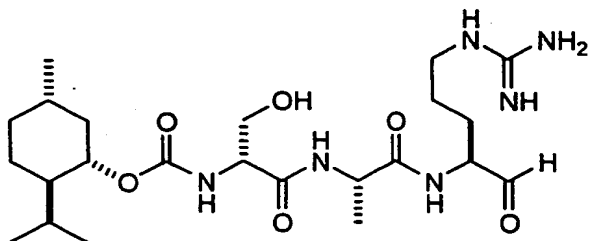


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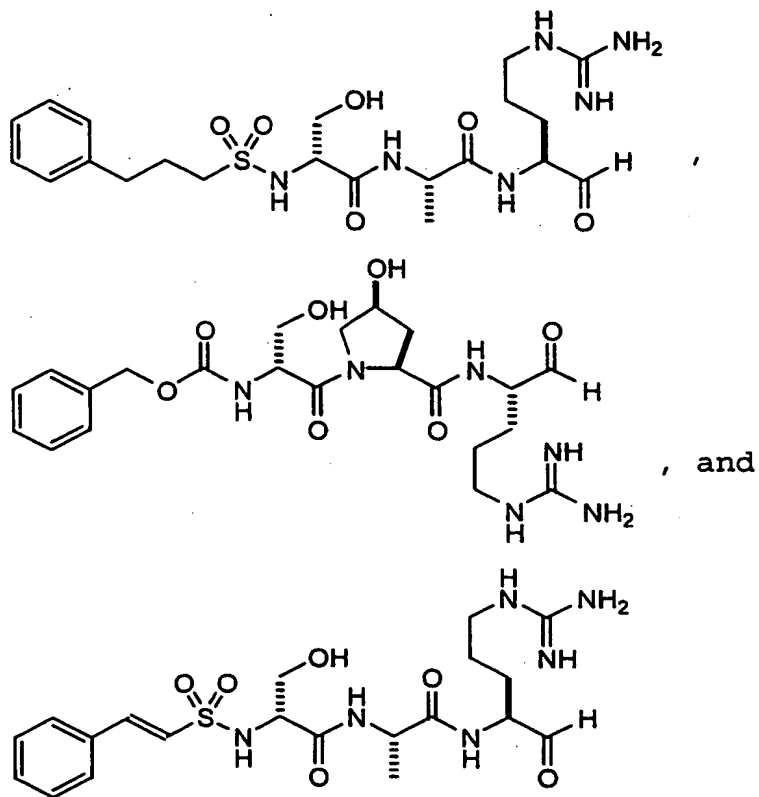
30. A compound according to claim 1 selected from the group consisting of



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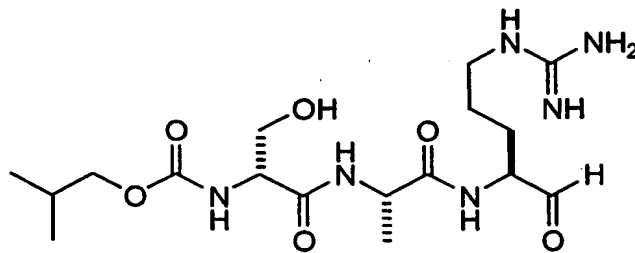


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31. A compound according to claim 7 wherein R_2 is
5 -CH₂OH and has the R configuration.

32. A compound of the formula:



or a pharmaceutically acceptable salt thereof.

33. A compound according to claim 1 wherein R_2 is
- $(CH_2)_2OA_2$ or - $CH(R_6)OA_2$.

34. A compound according to claim 33 wherein R_2 is
- $CH(R_6)OA_2$.

5 35. A compound according to claim 34 wherein R_6 is
selected from hydrogen, alkyl optionally substituted with Y_1 ,
and aralkyl optionally mono-, di- or tri-substituted with Y_1 ,
 Y_2 and/or Y_3 .

36. A compound according to claim 35 wherein R_2 is
10 selected to give a group at P3 selected from acyl and
carbonate esters of D-seryl.

37. A compound according to claim 36 wherein R_3 is
hydrogen.

38. A compound according to claim 37 wherein R_4 is
15 methyl or propargyl.

39. A compound according to claim 36 wherein R_3 and R_4
are selected together to give a group at P2 selected from
prolyl, pipecholy, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-
hydroxyprolyl and 3,4-dehydroprolyl.

20 40. A compound according to claim 1 wherein R_2 is
selected to give a group at P3 selected from acyl and
carbonate esters of D-seryl and R_5 is selected to give an
arginine aldehyde at P1.

41. A compound according to claim 40 wherein R_3 is
25 hydrogen and R_4 is methyl.

42. A compound according to claim 40 wherein R_3 and R_4 are selected together to give a group at P2 selected from prolyl, azetidine-2-carbonyl and 3,4-dehydroprolyl.

43. A compound according to claim 4 wherein R_2 is
5 - $(CH_2)_2OA_2$ or $-CH(R_6)OA_2$.

44. A compound according to claim 4 wherein R_2 is selected to give a group at P3 selected from acyl and carbonate esters of D-seryl.

45. A compound according to claim 29 wherein R_2 is H,
10 $-CH_3$, $-CH_2CH_3$, $-CH_2NH(X')(R_6)$ or $-CH(R_6)OH$.

46. A compound according to claim 29 wherein R_2 is
 $-(CH_2)_2OA_2$ or $-CH(R_6)OA_2$.

47. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and therapeutically
15 effective amount of compound of claim 1.

48. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 4.

49. A pharmaceutical composition comprising a
20 pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 12.

50. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 17.

51. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 21.

52. A pharmaceutical composition comprising a
5 pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 24.

53. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 26.

10 54. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of a compound of claim 30.

55. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically
15 effective amount of the compound of claim 32.

56. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 33.

57. A pharmaceutical composition comprising a
20 pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 40.

58. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering
25 to said mammal a therapeutically effective amount of a compound of claim 1.

59. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a
5 compound of claim 4.

60. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a
10 compound of claim 12.

61. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a
15 compound of claim 17.

62. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a
20 compound of claim 21.

63. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a
25 compound of claim 24.

64. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering

to said mammal a therapeutically effective amount of a compound of claim 26.

65. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in
5 a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 30.

66. A method of treating a condition which is ameliorated by inhibiting or decreasing urokinase activity in
10 a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 32.

67. A method of reducing or inhibiting blood vessel formation in a mammal in need of such treatment which
15 comprises administering to said mammal a therapeutically effective amount of the compound of claim 32.

68. A method according to claim 67 wherein said blood vessel formation is related to a pathologic condition.

69. A method of treating a condition which is
20 ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering to said mammal a therapeutically effective amount of a compound of claim 33.

70. A method of treating a condition which is
25 ameliorated by inhibiting or decreasing urokinase activity in a mammal in need of treatment which comprises administering

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to said mammal a therapeutically effective amount of a compound of claim 40.

Figure 1

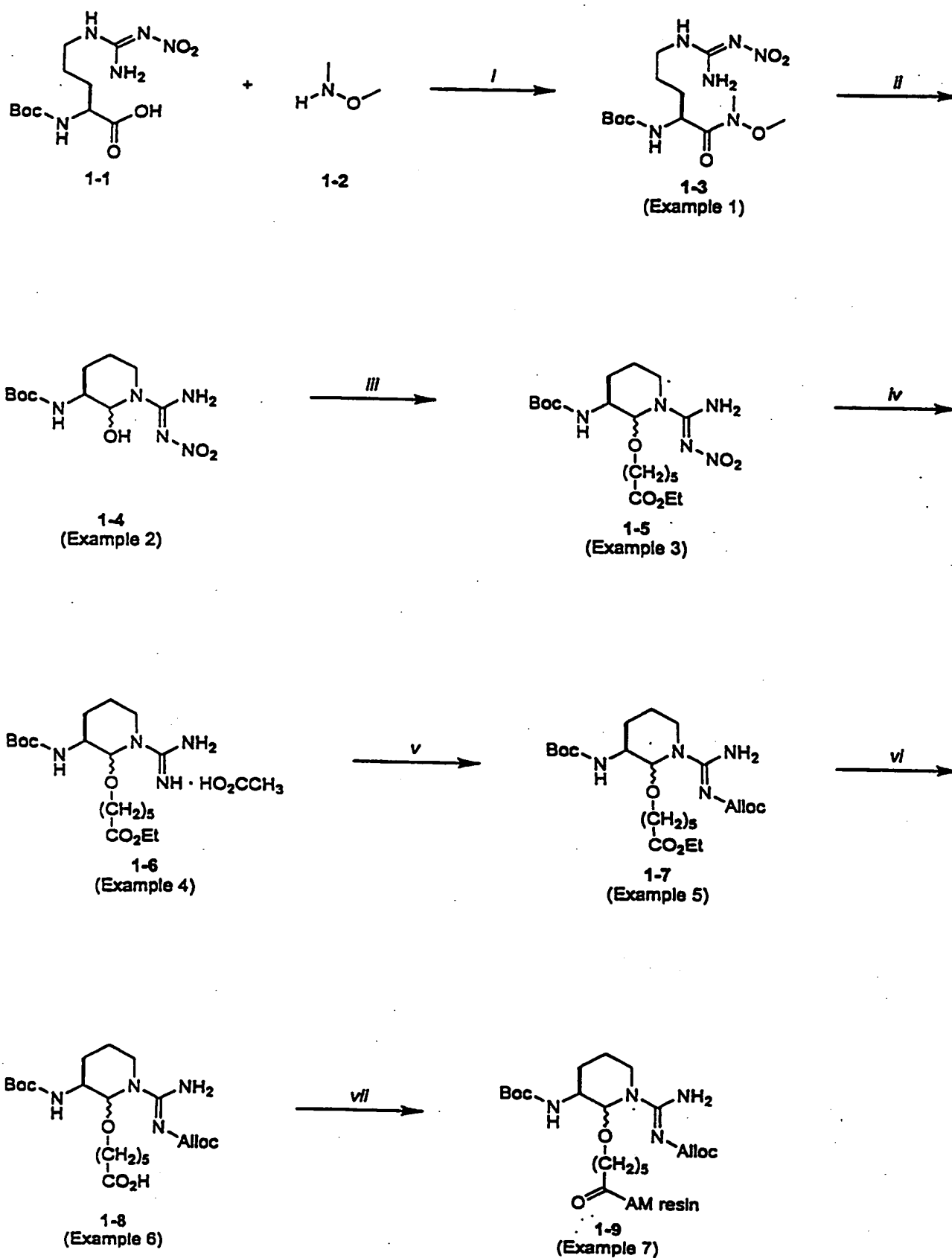


Figure 2

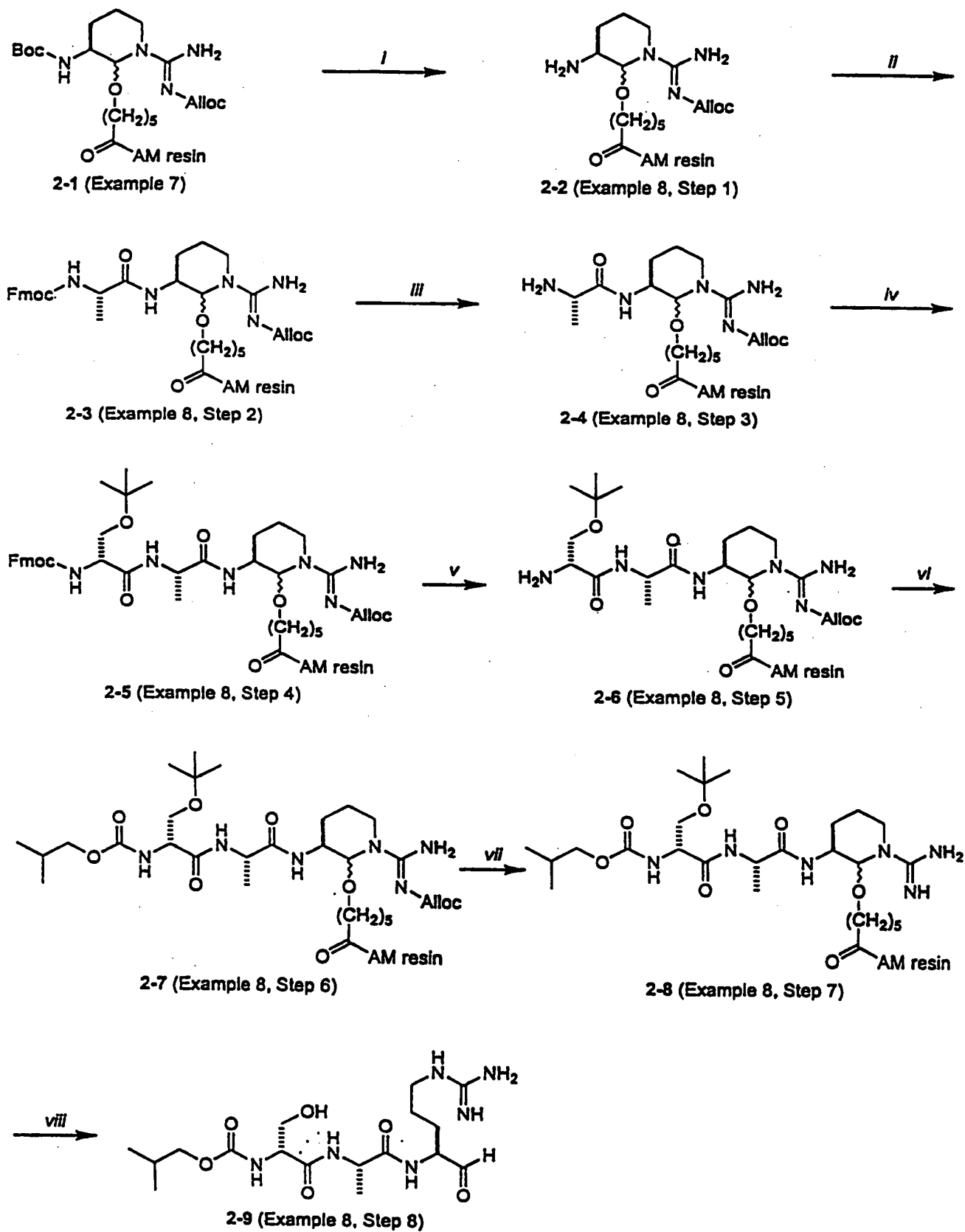


Figure 3

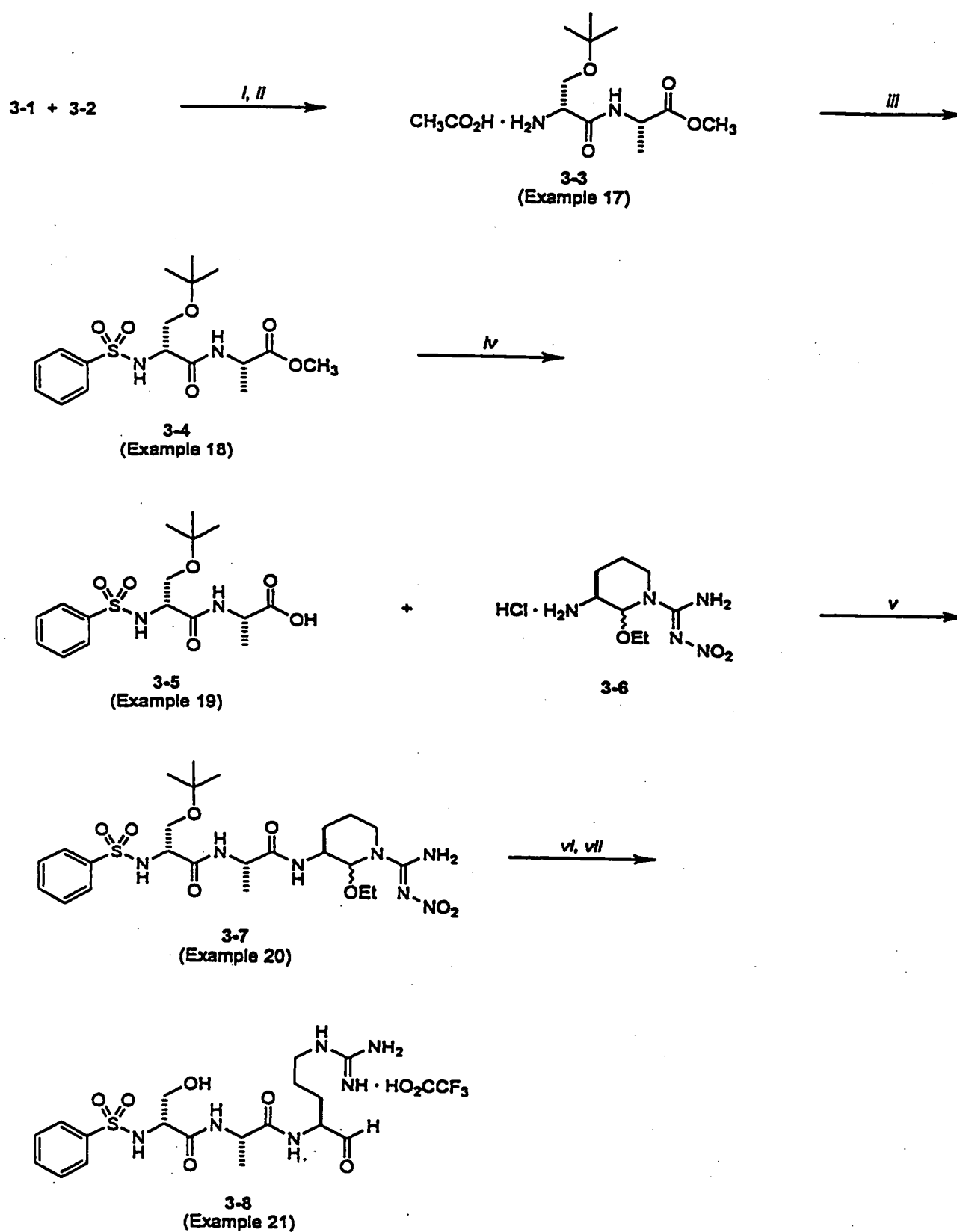


Figure 4

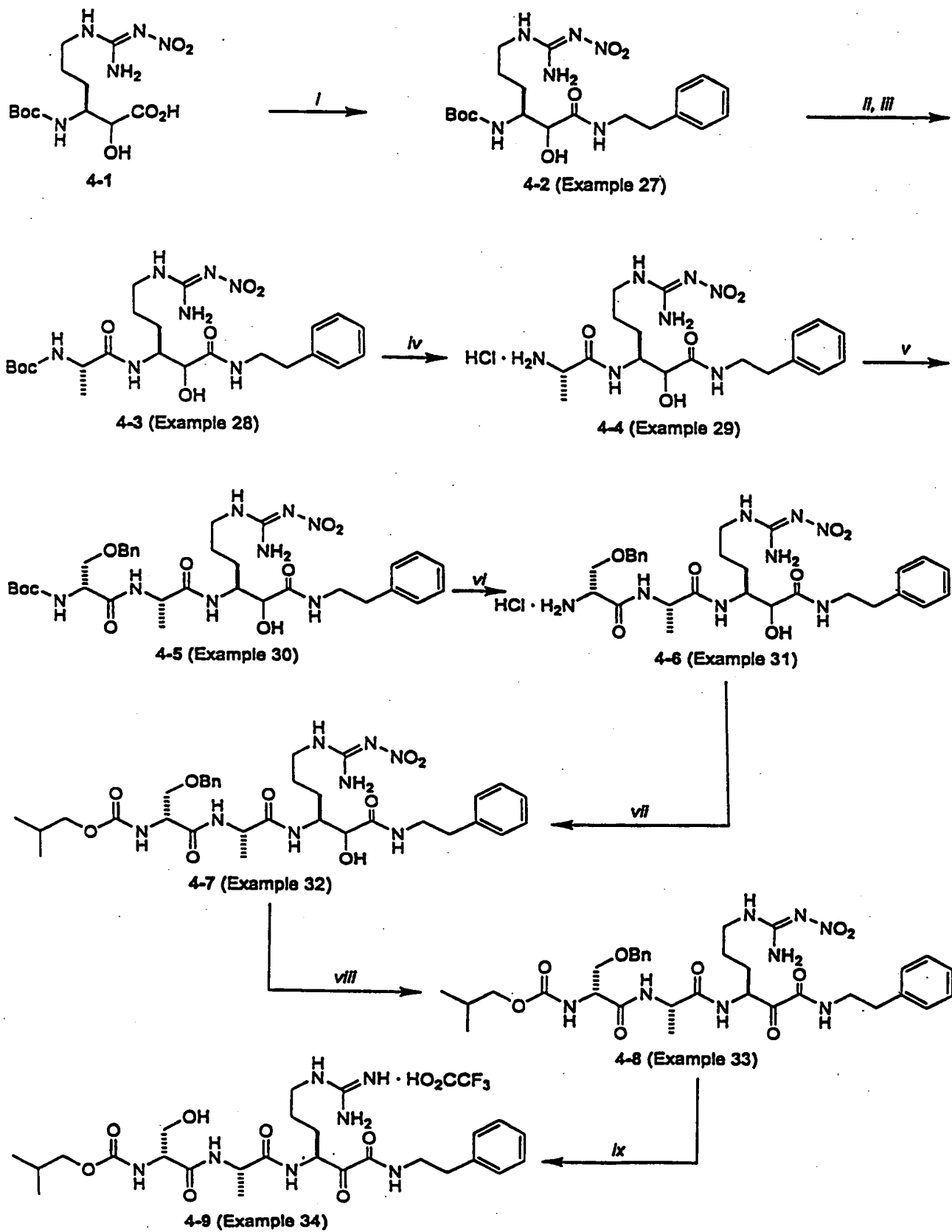


Figure 5

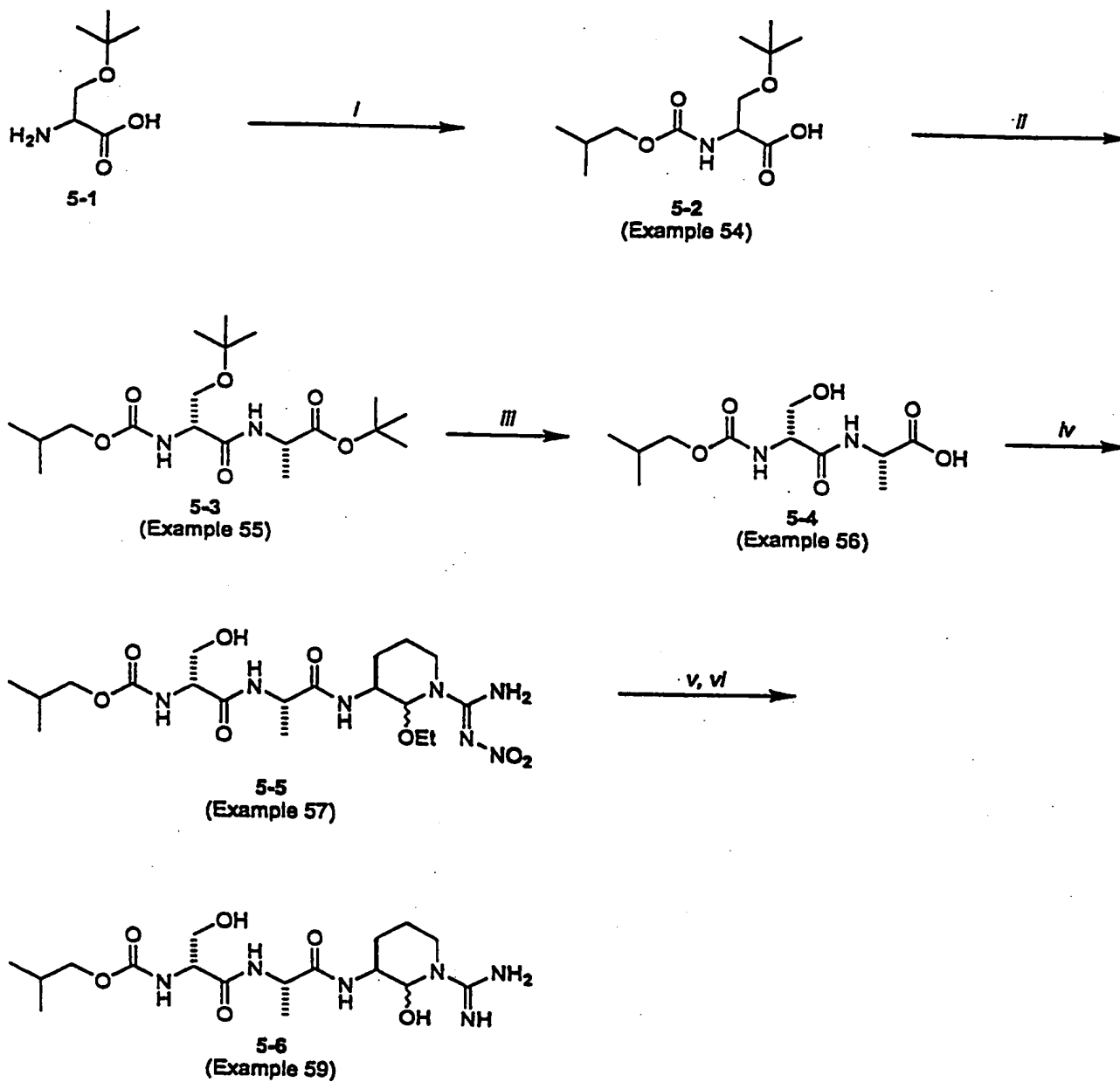


Figure 6

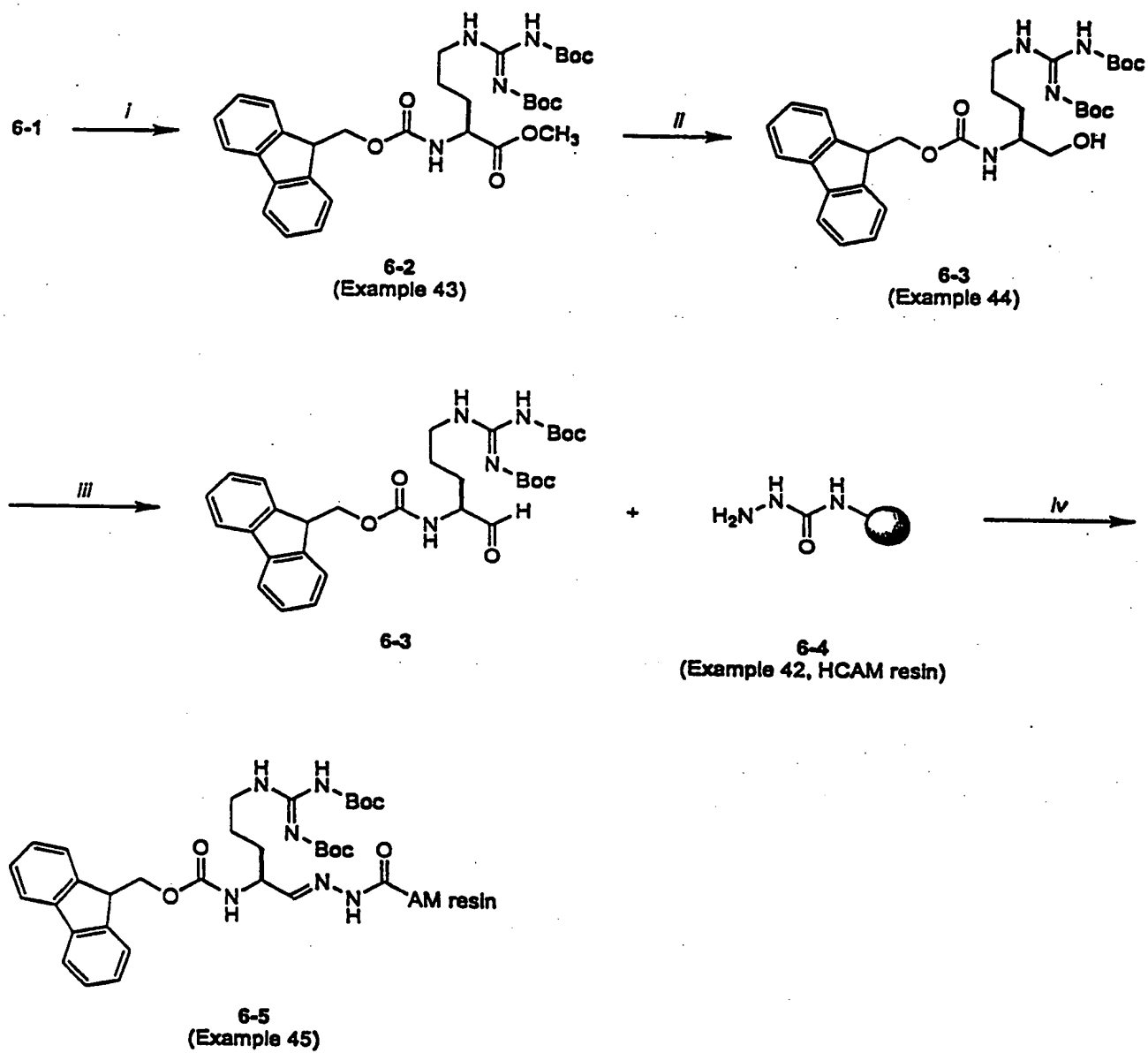


Figure 7

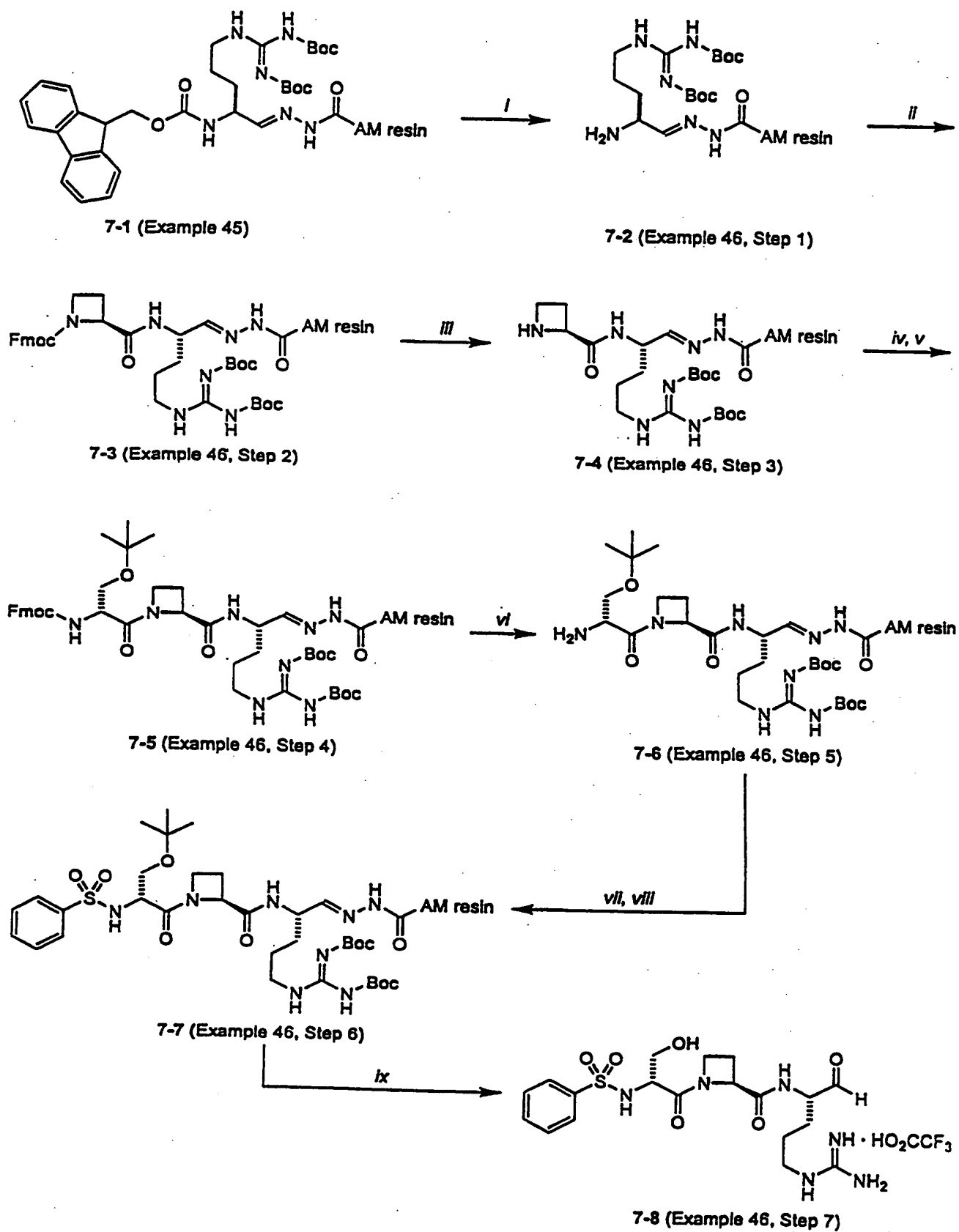


Figure 8

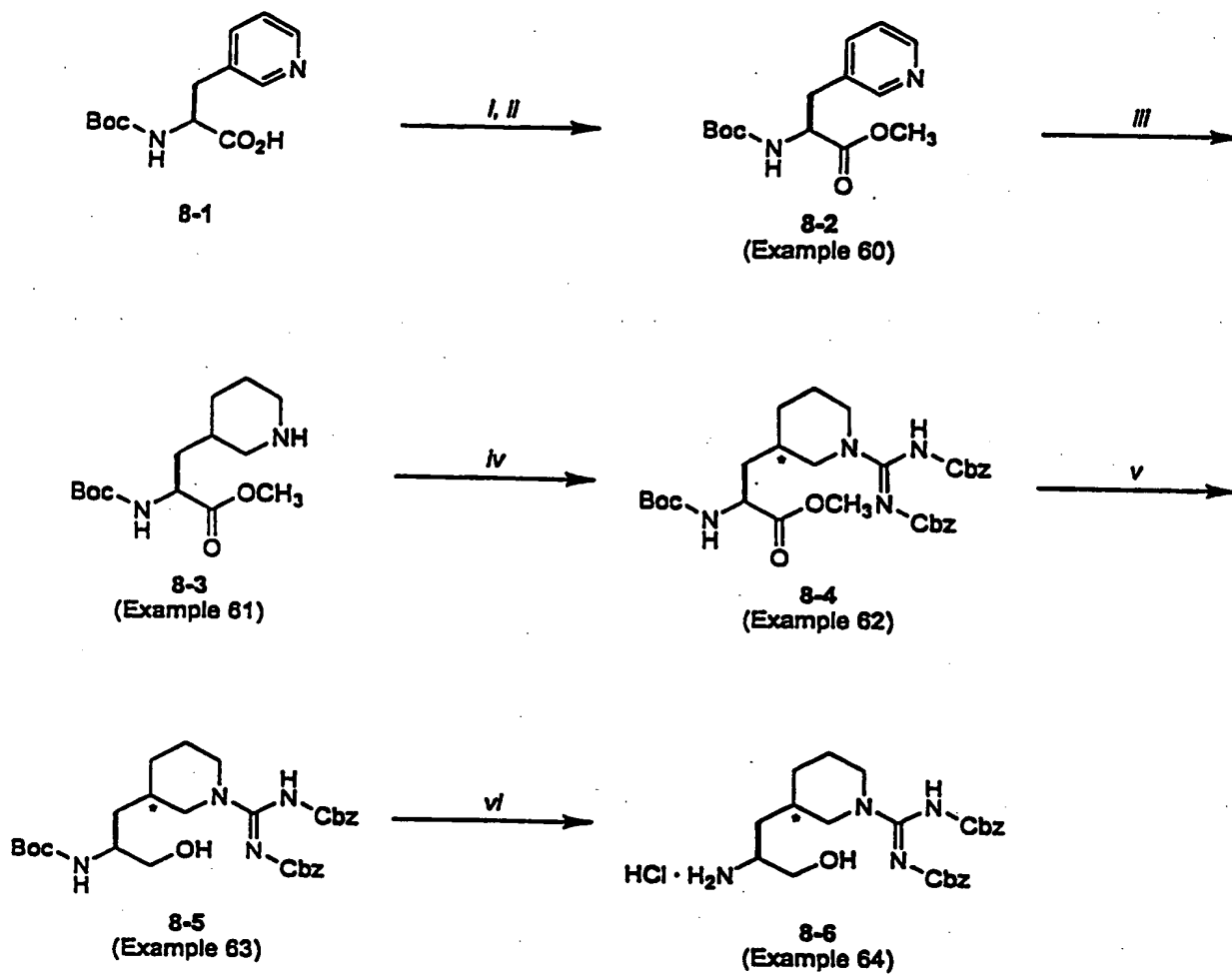


Figure 9

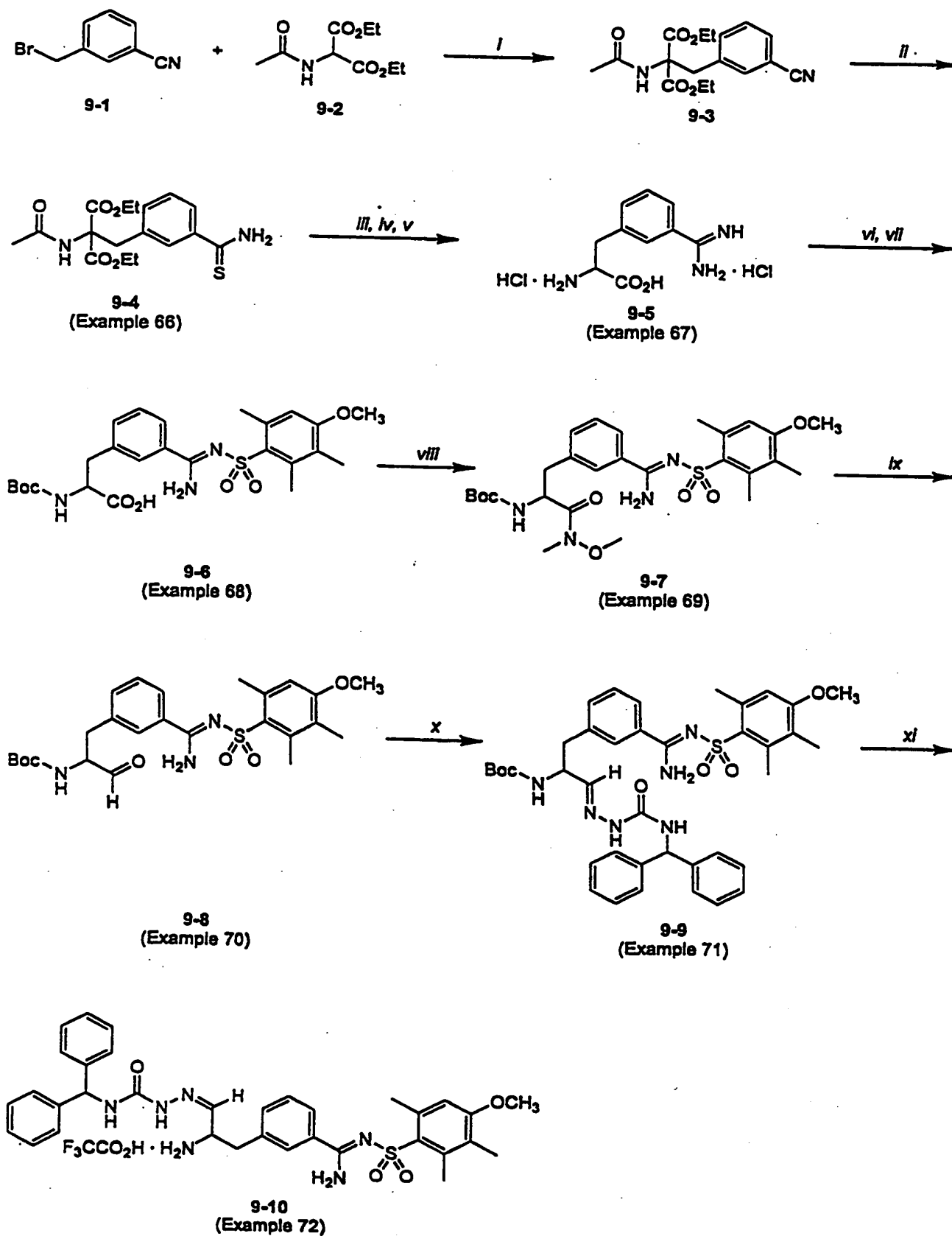
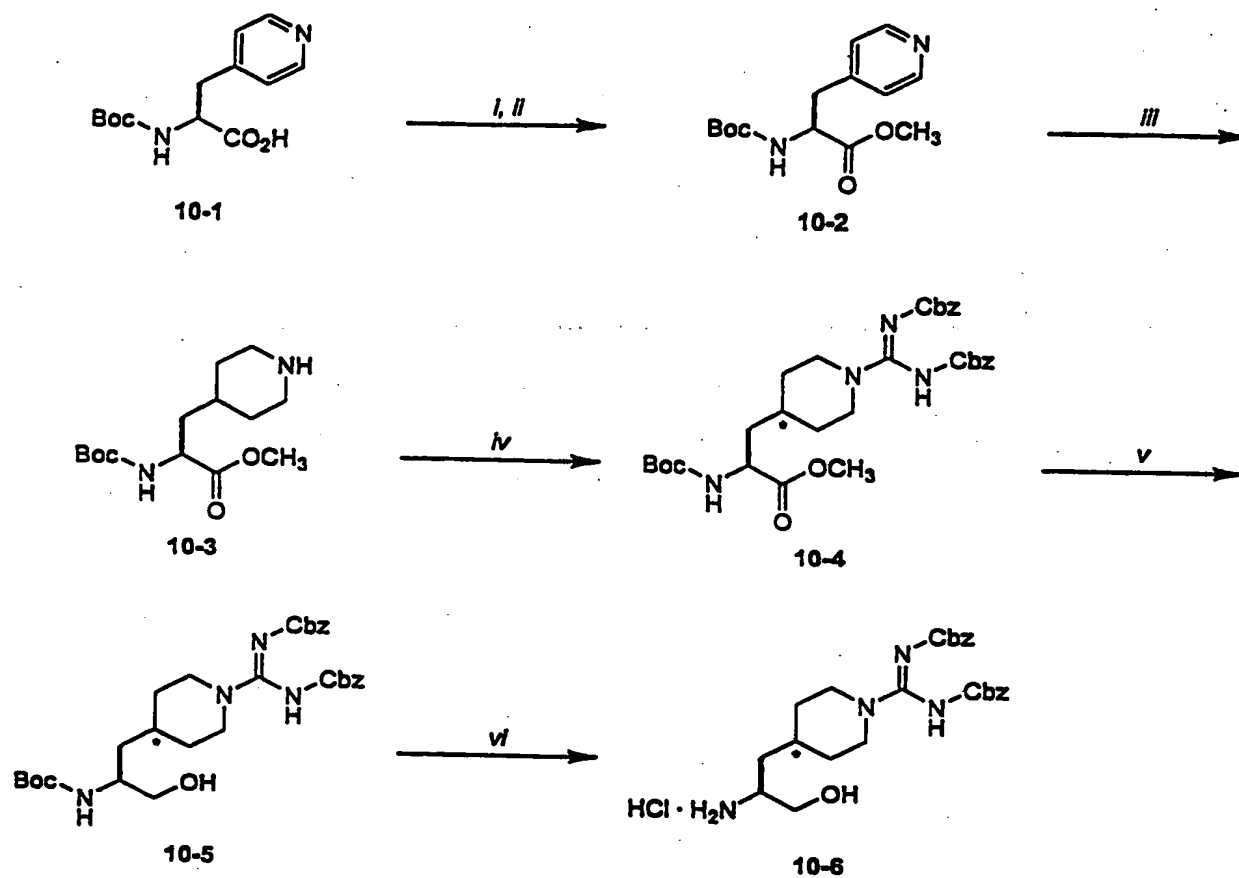
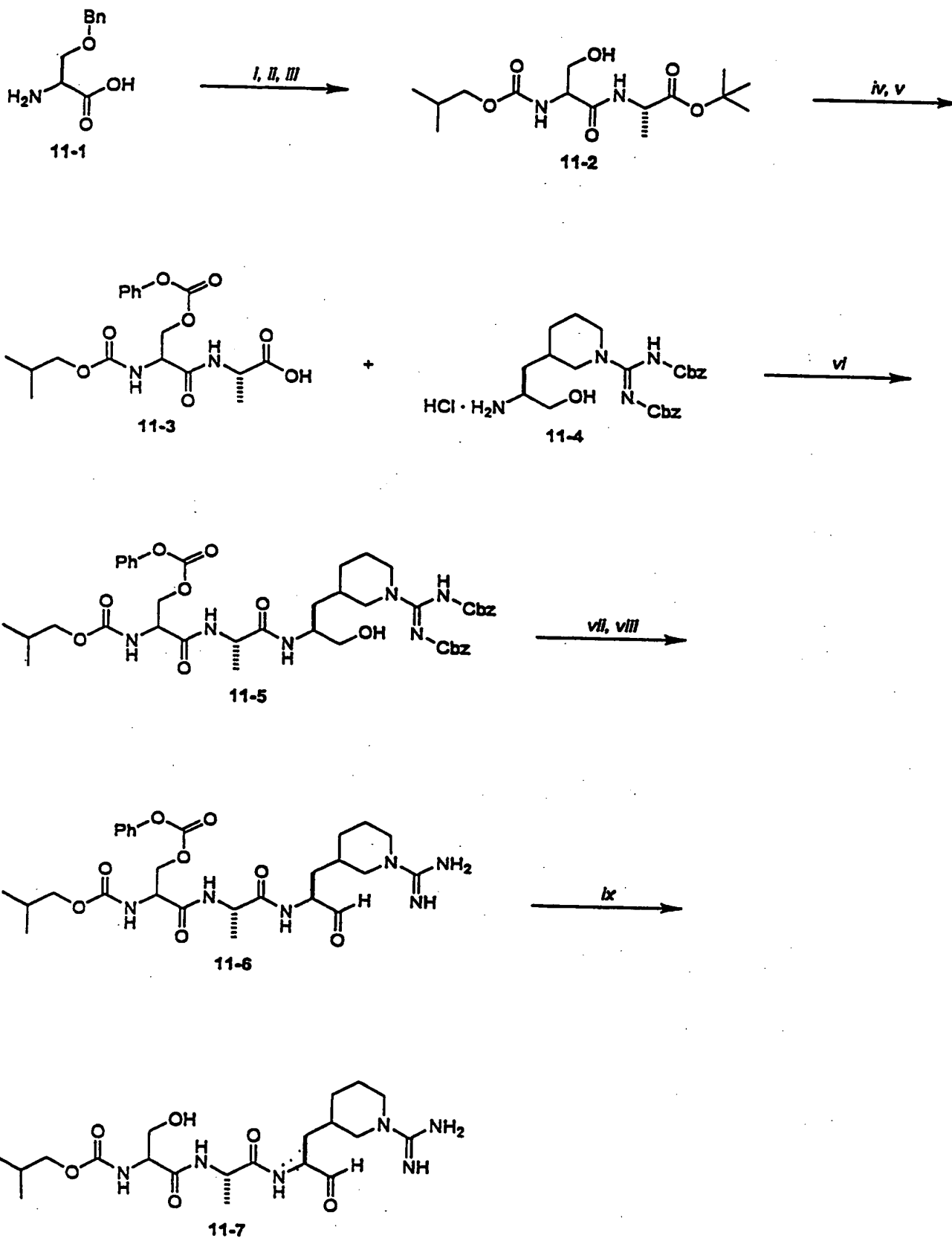


Figure 10



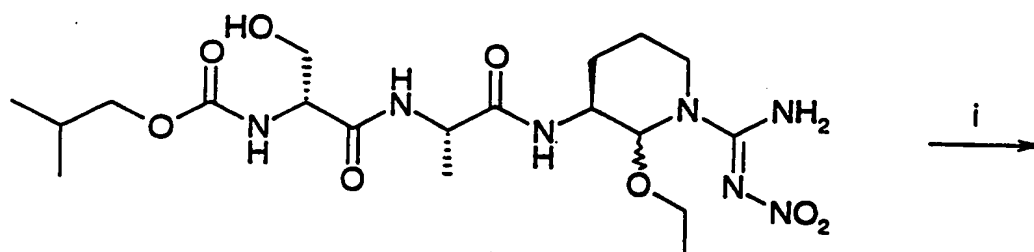


12-1 + **12-2** \xrightarrow{I} **12-3**

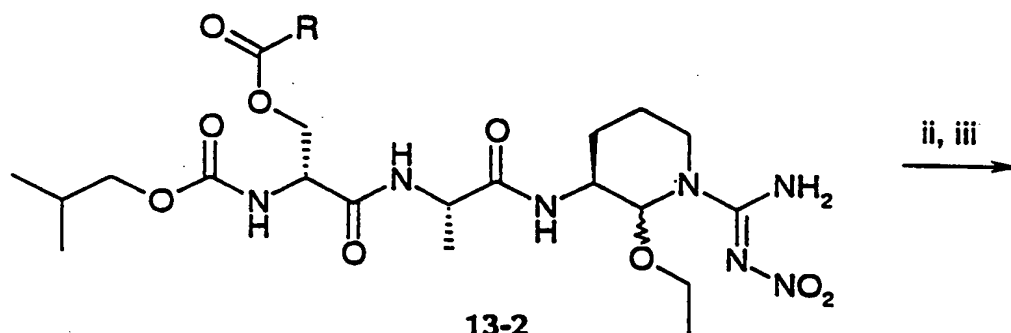
12-3 \xrightarrow{II} **12-4**

12-4 \xrightarrow{III} **12-5**

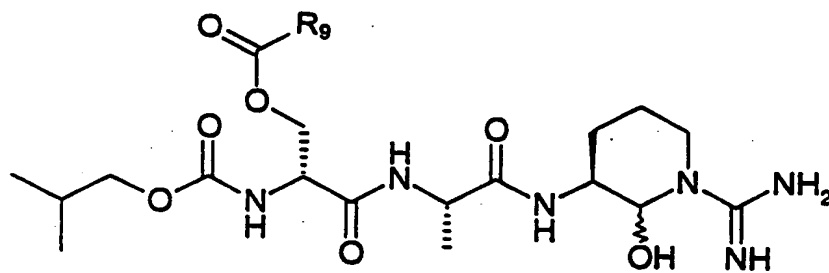
Figure 13



13-1
(Example 51)



13-2



13-3

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 5/062, 5/068, A61K 38/04	A3	(11) International Publication Number: WO 00/05245 (43) International Publication Date: 3 February 2000 (03.02.00)
(21) International Application Number: PCT/US99/16577 (22) International Filing Date: 22 July 1999 (22.07.99) (30) Priority Data: 09/121,921 24 July 1998 (24.07.98) US (71) Applicant (for all designated States except US): CORVAS INTERNATIONAL, INC. [US/US]; 3030 Science Park Road, San Diego, CA 92121 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BRUNCK, Terence, K. [US/US]; 4949 Quincy, San Diego, CA 92109 (US). TAMURA, Susan, Y. [US/US]; 8997 Gainsborough, San Diego, CA 92129 (US). (74) Agents: BIGGS, Suzanne, L. et al.; Lyon & Lyon LLP, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 20 April 2000 (20.04.00)
(54) Title: INHIBITORS OF UROKINASE AND BLOOD VESSEL FORMATION		
(57) Abstract <p>Novel compounds having activity inhibitors of urokinase and in reducing or inhibiting blood vessel formation are provided. These compounds have an arginine or arginine mimic aldehyde or an arginine ketoamide group at P1. These compounds are useful <i>in vitro</i> for monitoring plasminogen activator levels and <i>in vivo</i> in treatment of conditions which are ameliorated by inhibition of or decreased activity of urokinase and in treating pathologic conditions wherein blood vessel formation is related to a pathologic condition.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/16577

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K5/062 C07K5/068 A61K38/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PATEL E.A.: "Transition-state affinity chromatography of trypsin-like proteinases with dipeptidyl argininal ligands" BIOCHIM.BIOPHYS.ACTA, vol. 748, 1983, pages 321-330, XP002120735</p> <p>see the whole document</p> <p style="text-align: center;">--- -/--</p>	<p>1-5,7, 10, 13-18, 20,22, 23,27, 29,45, 47-50, 52, 58-61,63</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *&* document member of the same patent family

Date of the actual completion of the international search

5 November 1999

Date of mailing of the international search report

22.02.2000

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GROENENDIJK, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/16577

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BAJUSZ S ET AL: "DESIGN AND SYNTHESIS OF PEPTIDE INHIBITORS OF BLOOD COAGULATION" FOLIA HEMATOLOGY. INTERNATIONALES MAGAZIN FUER KLINISCHE UND EXPERIMENTELLE BLUTFORSCHUNG, vol. 109, no. 1, 1 January 1982, pages 16-21, XP000560955 see the whole document ---	1-5,7,9, 11-14, 18,20, 22,24, 25, 47-49,52
X	WO 94 08941 A (CORVAS INT INC) 28 April 1994 See especially compounds 27-35, examples 76 and 82 see the whole document ---	1,14-20, 22,24, 25,29, 45,47, 50,52
A	OSTROWSKI E.A.: "Selective inhibition of proteolytic enzymes in an in vivo mouse model for experimental metastasis" CANCER RESEARCH, vol. 46, August 1986, pages 4121-4128, XP002120736 see the whole document ---	1-5,7, 9-18,20, 22-25, 29,45, 47-50, 52, 58-61,63
A	US 5 153 176 A (ABE YOSHIHITO ET AL) 6 October 1992 cited in the application see the whole document ---	
A	US 5 508 385 A (ABE YOSHIHITO ET AL) 16 April 1996 cited in the application see the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 16577

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
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Remark: Although claims 58-70 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
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3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see additional sheet, subject 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-5,7,9-18,20,22-25,29,45,47-50,52,58-61,
63 (all partially)

Compounds containing the structures as defined in claim 1,
wherein R2 is H, CH3 or C2H5, their pharmaceutical
preparations and use

2. Claims: 8,21,26-28,30-44,46,51,53-57,62,
64-70(all complete), 1-7,9-20,22-25,29,45,47-50,52,
58-61,63(all part

Compounds containing the structures as defined in claim 1
wherein R2 is (CH2)2OH, (CH2)2OA2, CH(R6)OH, CH(R6)OA2,
their pharmaceutical preparations and use

3. Claims: 1-6,13-20,22-25,29,45,47,48,50,52,58,59,61,
63 (all partially)

Compounds containing the structures as defined in claim 1,
wherein R2 is CH2NH-X'-R6, their pharmaceutical preparations
and use

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/16577

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9408941 A	28-04-1994	US 5371072 A	06-12-1994
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